THERMAL EVOLUTION IN DROSOPHILA MELANOGASTER

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DECLARATION

I hereby declare that this thesis was composed by myself, and that the work described within it is my own except where stated in the acknowledgements.

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ABSTRACT

THERMAL EVOLUTION IN *DROSOPHILA MELANOGASTER*

The experiments reported in this thesis examined the effects of laboratory thermal selection on a range of characters in two sets of three replicate lines of *Drosophila melanogaster* , maintained at 16.5°C and 25°C for over four years.

The duration of developmental stages in males and females from both selection regimes was greater when reared at 16.5°C than when reared at 25°C. An evolutionary response of development times to temperature was also evident. Times to pupariation of 25°C selected larvae were longer than those of 16.5°C selected larvae, when reared at 16.5°C or 25°C. Times to adult eclosion were longer for 25°C selected lines compared to 16.5°C selected lines, when both were reared at 16.5°C, but the situation was reversed at 25°C. Pre-adult survival was higher in selected lines when they were reared at their own selection temperature. This is clear evidence of rapid thermal evolution and adaptation in the selected lines.

Larval growth patterns were also examined. Larvae from both selection regimes reached a higher weight, and took almost twice as long to develop, when reared at 16.5°C rather than 25°C. At both growth temperatures, 16.5°C selected larvae had higher mean weights at almost all sampling intervals in comparison to 25°C selected larvae. Starvation experiments showed that larvae from the thermally selected lines had higher critical weights for pupariation when reared at the temperature at which they had been selected compared to larvae from the other selection regime reared at the same temperature. Results suggested that 16.5°C selected lines may be allocating more nutrients to growth as opposed to somatic maintenance.

Larvae from thermally selected lines showed evidence of adaptation, with respect to larval competitive ability. Greater competitive ability was shown by larvae from each selection regime when they were reared and tested at the temperature at which they had evolved, compared to larvae from the other selection regime, reared and tested at the same temperature.

Development and evolution at 16.5°C both resulted in an increase in thorax length and wing area. The developmental and evolutionary responses of increased wing area were found to be due almost entirely to an increase in cell size rather than number. The similarity between the developmental and evolutionary responses of both body size and cell size was suggestive of adaptive phenotypic plasticity.

The life spans of both sexes and the fecundity and fertility of the females from both selection regimes were measured at both experimental temperatures (16.5°C & 25°C). Adult longevity was much greater when flies were reared and maintained at the lower temperature. The effect of selection temperature depended upon the temperature at which longevity was measured. Flies from both selection regimes showed signs of adaptation, having greater longevity when tested at the experimental temperature at which they had evolved, compared to flies from the other selection regime tested at the same temperature. Thermal selection had a significant effect upon fecundity. This character showed clear evidence of adaptation. Females from each selection regime showing greater fecundity at almost every sampling interval, when reared and tested at

the temperature at which they had evolved, compared to females from the other selection regime tested at the same temperature. The results suggest that there may be a trade-off between longevity and fecundity at the two temperatures. Alternatively, adaptation to one temperature may imply loss of adaptation to the other which the flies no longer encounter.

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Chapter 1 GENERAL INTRODUCTION

1.1 Clinal variation in relation to temperature

Geographic variation among populations of single species is well known and documented, (Stebbins 1950; Mayr 1963; Harper 1977). Studying widely distributed populations often reveals gradual geographic variation (a cline) in morphological and other traits that is correlated with environmental gradients, or with factors such as temperature. Such clinal variation has been reported in a wide variety of organisms (Endler 1977).

Clines related to a geographic gradient can occur both as a result of genetic change and because of direct environmental effects on development. Clinal variation has frequently been demonstrated to have a genetic component. For instance, loblolly pine (Pinus taeda) shows north-south clinal variation in size in eastern North America. The tallest individuals are found in the more northerly latitudes of the species range, with size decreasing gradually towards lower latitudes. Allen (1961) showed that seeds from different parts of the range along the eastern seaboard of North America from Virginia to Florida responded differently when planted in common conditions in Virginia. After six years growth he found that seeds from Virginia had the highest percentage survival and produced by far the tallest trees, followed in order (in both characters) by Louisiana, Georgia, Mississippi and Florida seed.

As pointed out by Endler (1977), the mere observation of clines, even ones with a genetic basis, cannot be considered, by itself, as a demonstration of effects of natural selection. The

occurrence of stochastic processes is difficult to exclude; clines could be caused by genetic drift followed by secondary contact, reduced gene flow or isolation by distance. A strong argument in favour of the adaptive interpretation of clinal variation is the existence of similar clines on different continents, in populations with different histories (Endler 1977). For instance, in D. melanogaster many traits (e.g. development time; wing length; ovariole number.) show similar clinal variation in Europe/Africa, Asia and Australia (David and Capy 1988).

Individuals from populations that occur in higher latitudes tend to be larger on average than individuals of the same species in lower latitudes (Mayr 1963). This trend was formulated by W. Bergmann in 1847; it is one of several "biogeographic rules" and is called Bergmann's rule. An extension of Bergmann's rule, known as Allen's rule, states that there tends to be a reduction in the size of protuberant parts of the body (such as legs, wings etc) in higher latitudes. Genetic differences in pigmentation occur between geographic populations of many species and their significance has been discussed extensively (Rensch 1960; Mayr 1963; Dobzhansky 1970; Merrel 1981). Populations which occur at lower latitudes are in general more heavily pigmented than those populations in higher latitudes. This phenomenon is known as Gloger's rule.

In most previous discussions, Bergmann's rule and Allen's rule have been attributed to selection for decreased surface area/volume ratio in endotherms in cold areas, with the assumption that this is associated with conservation of body heat (Futuyma 1986; Colinvaux 1986). However, clines in body size also occur in many ectotherms (Ray 1960), including small invertebrates such as Drosophila (Stalker and Carson 1947). These

small insects have a negligible thermal inertia and therefore adopt the temperature of their surroundings rapidly (Stevenson 1985), so rate of heat exchange cannot be implicated in these clines. It still remains a mystery why small ectotherms should follow these biogeographic rules. Bergmann's rule cannot simply be explained as an adaptive strategy for energy conservation, because although the per weight energy expenditure of large animals will be lower than that of smaller individuals, larger animals will still have a greater overall energy requirement. Large size may be related to a greater ability to store energy, perhaps of greater adaptive significance in harsh, variable or unpredictable environments (Thomas 1968). Even among endotherms, there is little evidence to suggest that heat regulation is the basis for Bergmann's rule (Cossins and Bowler 1987).

1.2 Clinal variation in body size in vertebrate ectotherms

Ray (1960) surveyed the available literature on ectotherms and found that 80% (of 40 species) conformed to Bergmann's rule. Ray stated that the untested assumption that this rule described an adaptation for heat conservation was confusing the issue. His conclusion was that Bergmann's rule is widespread in natural populations and applies equally to ectotherms and endotherms, supporting previous reviews (Schmalhausen 1949; Scholander 1955, 1956). Lindsey (1966) examined whether a trend towards larger sizes in higher latitude regions held when comparisons were made between whole taxonomic groups of ectotherms. He analysed data from 12,503 species of ectothermic vertebrates, and found a

general trend in fish and amphibians for the proportion of species with large adult sizes to increase from the equator to the poles.

Biogeographical rules were originally formulated on the basis of field collected material. Amphibians have been much studied in this regard. For instance, in the pacific tree toad Hyla regilla, the largest animals were found in the north and in the mountains, while the smallest were found in the more southern interior valleys and deserts of the Western USA (Jameson et al. 1973). Geographical variation in a number of other characters of amphibians has been described, (e.g climate and distribution of salamanders, Lotter and Scott 1977; life history characteristics in frogs, Berven 1982a, 1982b; variation in colour morph frequencies of cricket frogs, Gray 1983). For example, cricket frogs, genus Acris, are especially suited for studies of geographic variation because of their vast and ecologically diverse range. Body size in Acris species was found to be highly correlated with humidity, and to a lesser extent with temperature (Nevo 1973; Nevo and Yang 1979).

Amphibian larval development varies greatly throughout the geographic range of many species. For instance, in the wood frog Rana sylvatica, which has a geographic range of 10 million square kilometres in North America, larval periods are shortest in the north and increase in duration towards the south (Herreid and Kinney 1967; Berven 1982b). Larval periods are also known to increase with elevation (Berven 1982b). Prolonged larval periods and larger body sizes of both green frog, Rana clamitans, and wood frog, Rana sylvatica, larvae in mountain ponds were found to be directly related to the low water temperature (Berven et al. 1979; Berven 1982b). Although these studies document considerable

geographic variation in development patterns and body size, these can be explained by the effect of temperature during development (Berven et al. 1979; Berven 1982a). These studies do not provide any evidence to make a distinction between phenotypic plasticity and genetic differentiation

An attempt to look at whether trends observed in the wild were genetic or environmental in origin was made by Berven (1982b), who documented a clinal increase in larval body size of the wood frog, Rana sylvatica, with both latitude and altitude. Although environmental differences and non-genetic maternal effects accounted for a major proportion of these differences in size and larval periods, a significant part of this variation had a genetic basis. In order to find out to what degree the observed variation represented genetic differentiation between the populations, as opposed to environmental differences, reciprocal field transplant experiments were carried out (Berven and Gill 1983). There was genetic differentiation between the populations. Temperature variation between the two locations studied accounted for most (73-88%) of the observed phenotypic variation, although other variables correlated with temperature may also have been important. There was a strong genetic component to the thermal physiology of R. sylvatica; thermal tolerances differed significantly among larvae from different latitudes reared under standard laboratory conditions (Berven 1982b).

1.3 Clinal variation in invertebrate ectotherms

Geographical clines in body size also occur in many invertebrate ectotherms, with body sizes increasing both with

latitude and with altitude. Zooplankton from high latitudes developed more slowly, reached larger sizes and lived longer than their counterparts from warmer seas at lower latitudes (McLaren 1963, 1965, 1966). The hermit crab Pagarus hirsutiusculus, showed geographic variation in carcinization (the degree to which the body is protected by a tough exoskeleton) along the Pacific coast of North America (Blackstone 1989). Alaskan crabs were larger, with broader anterior carapaces and exhibited a greater degree of carcinization than more southerly populations. Alpatov (1929a) made a biometrical study of geographical variation in the honey bee, Apis mellifera, in Asia and North America. In the Asiatic populations, absolute body size and relative length of legs all showed a clear north-south cline on the Russian plains. When colonies were transplanted to North America they retained their morphological identity under new environmental conditions. Alpatov concluded from this that the differences between the populations were largely genetic in origin, rather than directly due to the environment. Krumbiegel (1936a, 1936b, 1936c) studied geographical variation in several species of Carabus beetles. He found clines for relative length of legs and antennae, with both of these appendages becoming shorter in the north.

Among invertebrates, many cases conforming to Gloger's rule are known, and it is generally found that pigmentation decreases when temperature or dryness increases (Merrel 1981). Dobzhansky (1933) studied geographical variation in elytral pigmentation of several species of Coccinellidae (lady beetles). He found clines for the general pattern of pigmentation, and size of spots, with the direction in agreement with Gloger's rule. Protection against heat and desiccation are the most likely

advantages of lighter colour, as less heat energy is absorbed per unit area of any given material when lighter coloured. Clines in body size and other aspects of phenotype have been studied most extensively in species of Drosophila (David and Capy 1988; Singh and Long 1992).

1.4 Clinal variation in body size in *Drosophila*

Geographical clines in body size have been reported in many Drosophila species. For example, Stalker and Carson (1947) compared 45 strains of D. robusta taken from different parts of its North American range. All samples were reared and measured under standard laboratory conditions so that any differences which emerged were primarily genetic in origin. The dimensions measured were head width, thorax length, tibia length, wing length and wing width. The latitude of the site of collection was negatively correlated with head and thorax length, but positively correlated with wing length and width. Clear north-south genetically determined clines in head width, thorax length, wing length and wing width therefore existed, with the northernmost population having the largest ratio of wing length to thorax length.

Similar trends were found in relation to altitude (Stalker and Carson 1948). Among strains of D. robusta coming from 1000ft to 4000ft, strains from the higher (colder) sites had larger legs and longer and wider wings. Head width or thorax length showed little or no correlation with altitude, unlike the latitude study (Stalker and Carson 1947). In a further study (Stalker and Carson 1949), a single population was sampled at different times of the year and showed significant seasonal variations in all measurements. These

differences were such that, during spring, the population tended to resemble the northern and high altitude populations. During the summer, the population gradually changed towards the proportions of southern and low altitude populations. However, the trend that was recorded was very slight and the numbers sampled were rather low. Stalker and Carson suggested that the differences were adaptive responses to climatic differences over the species range, with both affected traits showing both spatial and temporal genetic variation. Both the latitudinal and altitudinal clines in D. robusta suggest a strong tendency for wings and legs to change together and for head and thorax size to change together. The second pair of characters showed no correlation with the first.

A distinct north-south cline in wing size among eight British populations of D. subobscura was found by Prevosti (1955). Animals were reared and measured under standard conditions, and wings were largest in the northernmost populations, while all the British populations had larger wings than a population from Barcelona. Misra and Reeve (1964) examined the variation in body dimensions among twelve populations of D. subobscura, coming from an area spread over the species' range from Scotland to Israel and kept under standard laboratory conditions. The measurements were essentially the same as those of Stalker and Carson (1947), with some minor differences in procedures, but the clinal pattern differed somewhat from that reported for D. robusta (Stalker and Carson 1947). Correlations of size with latitude were all positive and very high, with those for males being higher than for females, and both higher than for D. robusta. Correlations were higher for wing size and tibia length than for head width and thorax length. The general picture was of a very uniform geographical cline in all

the traits measured, each increasing with increasing latitude. Wing length and width followed opposite trends in the two species, as they were negatively correlated with latitude in D. robusta, but positively in D. subobscura. D. subobscura showed a 1% change in both wing dimensions and in femur length for every 3° change of latitude. D. robusta required about 5° change to bring about a 1% change in wing dimensions or head width. These data suggest that different species do not react in the same way to the selective agents responsible for clines in body dimensions. From the results of Misra and Reeve (1964), changes in head width and thorax length are correlated together, while change in wing length is associated with change in leg length only. Changes in head width and thorax length produce patterns characteristic for Bergmann's rule. The other correlated changes in wing and leg length alter the relative lengths and proportions of protuberant parts of the body, producing a pattern corresponding to Allen's rule in D. robusta, but not in D. subobscura.

Wing length and thorax length in the sibling species D. melanogaster and D. simulans collected from Lebanon, Egypt and Uganda were examined by Tantaway and Mallah (1961). Stocks were kept for several generations in standard conditions and then reared at a wide range of temperatures. The Lebanon population had greater wing length and thorax length at all temperatures than did the Egyptian and Ugandan flies. Lebanese flies showed less phenotypic variance of wing length than did the Egyptian or Ugandan populations at all temperatures. These differences between the geographical populations were more marked in D. melanogaster than in D. simulans.

Laboratory populations of D. melanogaster from tropical Africa and from France were established by David and Bocquet (1975a, 1975b) under uniform laboratory conditions at 25°C. The French strains had greater fresh weights for both males and females than the Afrotropical flies, and flies from intermediate latitudes were intermediate in weight. In a parallel study (David and Bocquet 1975a), American tropical strains (Guyana, Colombia, Ecuador) were compared to those from North America (USA and Canada). Large and highly significant differences in male and female fresh weight were found between the two groups, North American strains had greater fresh weights than tropical American strains, indicating that a latitudinal cline for these characters also existed in the wild American populations.

Twelve different populations of D. melanogaster from various altitudes were examined by Louis et al. (1982) for genetic differentiation. Strains were collected in Cameroon, West Africa at 180m, 1000m and 2000m. Another population, from East Africa was collected between 1000-1500m. All strains were reared at 25°C and the following traits were measured: fresh weight, wing length, wing breadth and thorax length. All the traits showed a positive correlation with altitude, with individuals from the 2000m population larger than those from 1000m, which were larger than those from 180m population.

1.5 Clinal variation in life history in *Drosophila*

1.5.1 Clinal variation in development times

Development times at 25°C of twelve different strains of D. melanogaster from different altitudes in Cameroon, West Africa

were recorded by Louis et al. (1982). Development time showed an decrease with altitude, from 213.4 hours through 209.1 hours, to 207.4 hours, for the low medium, and high altitude populations respectively.

Significant differences were detected for development time in three populations of D robusta (coming from 1000ft, 1360ft and 2000ft) reared at 15°C, 20°C and 25°C (Etges 1989). The 1000ft population was almost a full day slower than the 1360ft and 2000ft populations in development time at 15°C, but had a significantly faster development time than the other populations at 2 0°C and 25°C. That fact the low altitude population developed faster than the other populations at high temperatures, but not at colder temperatures, may reflect adaptation of the low altitude population to its environment, which is characterised by high ambient temperatures.

1.5.2 Clinal variation in viability

Pre-adult viability in Drosophila also shows latitudinal and altitudinal variation. Pioneering work by Timofeef-Ressovsky (1933) on Drosophila funebris measured the relative larval viability of different strains from Europe and Russia in the laboratory, and showed that strains from the southern localities had higher viability at high temperatures than at lower temperatures, while strains from the northern areas showed the reverse relationship.

Populations of D. melanogaster and D.simulans from Lebanon, Egypt and Uganda were examined by Tantaway and Mallah (1961) for differences in pre-adult viability, at a range of temperatures between 10°C and 31.5°C. The Lebanon population showed greater

pre-adult viability than all the other populations studied, at all but high temperatures. By comparison, the population from Uganda showed greater pre-adult viability only at high temperatures i.e. their original climatic conditions. This greater viability at most temperatures may have reflected adaptation to wider temperature extremes in the Lebanon population.

Twelve populations of D. melanogaster collected from different altitudes in Cameroon, West Africa showed significant differences in percentage pre-adult mortality when measured under standard laboratory conditions at 25°C (Louis et al. 1982).

Pre-adult viability in D. robusta was examined by Etges (1989). Testing three populations (from1000ft, 1360ft and 2000ft) at 15°C, 20°C and 25°C, he found significant differences in egg-to-adult viablity between populations at all temperatures. The 2000ft population exhibited lower egg-to-adult viability than either the 1000ft or 1360ft populations, at all three temperatures.

1.5.3 Clinal variation in fecundity

A relationship between latitude of origin and female fecundity at different temperatures was demonstrated by Dobzhansky (1935) in the two closely related species, D. pseudoobscura and D. persimillis. In both species, strains from southern locations had higher fecundity at high temperatures than at low temperatures, while populations from the northern locations had higher fecundity at low temperatures than at high temperatures.

In the examination of altitudinal variation in life history charcters in D. robusta, Etges (1989) showed that over all the three temperatures studied, adults from the 1000ft population matured

more slowly than adults from the 1360ft and 2000ft populations. Age at first reproduction was greater in the 1000ft population.

A smooth altitudinal cline in fecundity was described by Devaux and Lachaise (1987) in D. yakuba in the Ivory Coast, measured under laboratory conditions at 25°C. A decrease in fecundity was found in flies from the higher latitudes. This was in contrast to D. teissieri, whose fecundity increased with altitude of origin, when measured in the laboratory at 25°C (Devaux and Lachaise 1987). Female fecundity in D. melanogaster varies both latitudinally and altitudinally in field conditions, generally decreasing with increasing latitude and altitude (Lemeunier et al. 1986). There is a genetic component to this temperature-related variation in fecundity, because flies from different latitudes and altitudes exhibit differences in fecundity and fertility when reared in controlled laboratory conditions at a standard temperature (David and Capy 1988; Capy et al. 1983,1986; Lemeunier et al. 1986).

Ovariole number in laboratory populations of D. melanogaster from tropical Africa and from France were examined by David and Bocquet (1975a, 1975b), under uniform laboratory conditions at 25°C. Females from the French strains were found to have more ovarioles than the African females, with flies from intermediate latitudes having an intermediate ovariole number. Similarly, comparison of tropical American and North American populations revealed highly significant differences between these groups in ovariole number. The adaptive significance of ovariole number can be explained in relation to traits such as reproductive capacity. Small ovariole number has been shown to be correlated with low reproductive potential in D. melanogaster (David 1970;

David and Bocquet1975a). The regularity, parallel nature and repeatability of these clines makes a very powerful case for climatic adaptation being the root cause (David and Capy 1988).

1.6 Clinal variation in other characters in *Drosophila*

Abdominal and sternopleural chaetae numbers in different strains of D. melanogaster have been shown to vary clinally in relation to altitude (Louis et al. 1982). Measured under laboratory conditions at 25°C, abdominal chaetae numbers increased with altitude, while sternopleural chaetae numbers decreased.

In D. melanogaster there is an area on the top of the thorax which shows variable pigmentation, depending on the environment. This pigmentation has a characteristic shape and is known as the thoracic trident. David et al. (1985) studied thoracic trident pigmentation in relation to average annual temperature at the site of collection. In flies grown at standard temperature, he found a very steep cline towards increased pigmentation at higher latitudes. Most latitudinal clines in Drosophila are expressed over a long geographic range. However, while latitudinal variation in trident pigmentation occurs between 30° and 50° of latitude on different continents, the trident cline exhibits its main effect over less than 1500km and is very steep. Wild living D. simulans do not exhibit a thoracic dark trident, but a pigment does appear in wild collected flies reared at low temperatures (Capy et al. 1988). In a study of 26 populations reared under controlled laboratory conditions, a latitudinal cline in trident pigmentation was expressed only at a developmental temperature of 17°C, and not at 2 5°C in D. simulans.

Climatic environmental stress differs considerably with latitude. The annual cycle of temperatures seen in temperate zones provide one of the greatest sources of environmental stress. Variations in desiccation tolerance have been studied in Australian populations of D. melanogaster from different latitudes, and temperate populations were found to be more resistant to stress than populations from subtropical regions (Parsons 1978a, 1978b, 1980, 1983; Stanley and Parsons 1981; Davidson 1988, 1989). Da Lage et al. (1990) studied starvation tolerance and desiccation tolerance in three populations of D. melanogaster from France, Tunisia and the Congo. The Tunisian populations were more resistant to desiccation than the French or Congo populations. However, the Congo population was about twice as resistant to starvation as the French or Tunisian populations. Afrotropical flies which live in hot and humid conditions are poorly protected against desiccation but need protection against starvation because of their high metabolic rate, due to high ambient temperatures.

Afrotropical strains of Drosophila species generally experience stable conditions, so there would be little selective pressure to adapt to wide temperature extremes. Endemic tropical species are usually stenothermic, with narrow thermal tolerance ranges, but cosmopolitan species such as D. melanogaster and D. simulans have bigger geographical ranges, probably partly because of their wide themal tolerances (David and Clavell 1966; Cohet et al. 1980).

Genetic latitudinal clines in alcohol dehydrogenase (Adh) allele frequency in D. melanogaster have been observed in North America, Asia and Australia (Johnson and Schaffer 1973; Vigue and Johnson 1973; Voelker et al. 1977; Oakeshott et al. 1982;

Anderson et al. 1987). The observed negative correlations of Adh^S frequency with latitude is possibly associated with temperature, because of the higher thermal stability of Adh^S (Vique and Johnson 1973). Interpretation of the Adh cline is complicated by the fact that the common cosmopolitan inversion In(2*L*)t, which also shows latitudinal clines in three continents (Mettler et al. 1977; Inoue and Watanabe 1979; Knibb et al. 1981) is associated with alleles of Adh (Watanabe and Watanabe 1977; Inoue et al. 1981). There is also a positive correlation between latitude and the frequency of the $\alpha Gpdh^S$ allele in North America, Asia and Australia (Johnson and Schaeffer 1973; Oakeshott et al. 1982). This cline is believed to be due to temperature related genotypic differences in biochemical processes, such as enzymatic activity and function (Alahoitis et al. 1977). Ethanol tolerance increased linearly with latitude in D. melanogaster, between Afrotropical and European populations and between tropical American and North American populations, enabling flies to utilize more alcoholic substrates, such as fermenting grapes. Tolerance was lower in D. simulans and was independent of latitude (David and Bocquet 1975a; 1975b). It seems that alcohol tolerance is directly related to Adh activity. The fast allele of Adh has higher enzymatic activity, and exhibits a latitudinal cline, with frequency increasing with latitude (e.g. Vigue and Johnston 1973).

In D. melanogaster, latitudinal clines for several different inversions have been observed in three different continents. Inversions are thought to play an important role in adaptation to environmental stress (Dobzhansky 1970; Lewontin et al. 1981). Marinkovic' et al. (1969) provided evidence in D. pseudoobscura that cold resistance is associated with certain chromosome

inversion types. Altitudinal clines of chromosomal arrangements and allozyme frequencies are also known on several continents (Dobzhansky 1943, 1948; Voelker et al. 1978; Pipkin et al. 1976). For a cosmopolitan species like D. melanogaster, the existence of so many parallel clines over different continents is a very powerful argument in favour of their adaptive significance (Endler 1986).

Clines in many chromosomal arrangements in D. subobscura have been documented in the Old World and the New World (Brncic et al. 1981; Prevosti et al. 1985, 1988, 1990). A study of the recent colonisation of Western South America and North America by D. subobscura (Prevosti et al. 1985, 1988) has shown that three years after the species was first detected in South America, and four years after first detection in North America, latitudinal clines had already been established in chromosomal arrangement frequencies. The similarity of these clines to Old World clines provides very strong evidence for the adaptive nature of this polymorphism, with respect to environmental factors that change with latitude.

Natural populations of Drosophila species are different in several respects from laboratory populations. For instance, they are far more variable in size (e.g Sokoloff 1965, 1966; Tantaway 1964; David 1979; David and Capy 1988). In a comparison of wild- and laboratory-reared D.subobscura McFarquhar and Robertson (1963) showed that wild D.subobscura were smaller and very much more variable than laboratory reared flies (see also Coyne and Beecham 1987 for D. melanogaster). Similarly, David et al. (1979) examined the phenotypic variability of wild-collected D. melanogaster. They found that wild flies were much smaller and far more variable. Average temperatures in France were lower

than in the laboratory, which should have produced bigger wild flies (see section 1.8 on the environmental effects of temperature). Since the wild flies were smaller it would seem that nutritional factors were responsible for the reduced size. An increase in larval competition in the wild would produce a reduction in food resources (Bakker 1961; Atkinson 1979) and this is known to reduce size (Atkinson 1979). McFarquhar and Robertson (1963) developed this hypothesis to account for their results. In the wild, the heritability of most traits is very low compared to the strongly positive heritabilities usually seen in laboratory populations (Coyne and Beecham 1987). The fact that observable clines do persist in the wild is therefore testimony to strong selective forces

1.7 Evolution of Drosophila body size and life history in relation to laboratory temperature.

That discernable latitudinal clines occur repeatedly in nature is taken by many workers to be a clear indication of the importance of temperature as a selection pressure (e.g. Berven 1982a, 1982b; Berven and Gill 1983; David et al. 1985; Da Lage et al. 1989). However, temperature is in fact only one of many candidate independent variables. Laboratory populations maintained at different temperatures provide a means of assessing the selective effects of temperature on body size with fewer complications from other environmental factors (e.g. rainfall and humidity) that vary clinally with temperature in the wild. It is also possible to control for other variables (e.g. food quality and quantity or interspecific competition) that may affect body size (Economos and Lints 1984a, 1984b; Atkinson 1979). In addition, D. melanogaster does not

necessarily experience the full range of ambient temperatures in the wild, because it can restrict its thermal environment by habitat selection (Jones et al. 1987). Given that the precise thermal environment occupied by Drosophila in the wild is therefore uncertain, the importance of controlled evolution experiments under standard laboratory thermal regimes becomes clear. Such "laboratory natural selection" (Huey et al. 1991) has been very successfully exploited to study adaptation to thermal regime in E. coli (Lenski et al. 1991; Bennett et al. 1992).

Drosophila thermal evolution experiments have in general revealed a genetic component to size change; when reared at a common temperature, flies from colder selection regimes are larger than those from warmer regimes. For instance, Anderson (1966) studied experimental populations of D. pseudoobscura established by M. Vetukhiv in 1958, which were cultured at 16, 25 and 27°C. After one and a half years there was no evidence for divergence in body size, as measured by wing length. However, after six years a divergence was evident, with 16°C flies being genetically determined for much larger wing length than the 25°C and 27°C populations (between which there were no significant differences). Anderson (1973) reported on these same populations 12 years after their isolation. The correlation between environmental temperature and body size was even clearer, with a significant difference in wing length between all three populations. The genetic divergence accounted for about 40% of the total phenotypic variation in wing length over the temperature range 16°C to 27°C (Anderson 1973). As this association between wing size and temperature in laboratory populations is similar to that observed in natural Drosophila populations, it seems likely that

temperature-related selective forces acting on wing size in the laboratory are much the same as those acting in the wild. Chance divergence can be ruled out because the pattern was repeated in the replicates of each subpopulation. Temperature was the major environmental factor manipulated, thus the morphological differentiation observed is likely to have been attributable to it. However, flies were cultured in population cages, and other factors such as population density and food supply may also have changed with temperature.

Lints and Bourgois (1987) measured three quantitative traits (development time, thorax length and wet weight) under standard rearing conditions at 25°C and 28°C, at regular intervals over a six year period in subpopulations (no replicates) of D. melanogaster, cultured in cages at 21°C, 25°C and 28°C. They found that significant differences in duration of development and in thorax length appeared very early, only 20 weeks after isolation of the subpopulations at the three temperatures. Differences in wet weight were apparent after 36 weeks separation. This confirmed previous results with Vetukhiv's populations (Ehrman 1964; Anderson 1966; Matzke and Druger 1977), except that differentiation arose much earlier than previously reported. When tested at 25°C, the flies from the 21°C cage were larger than the flies from the 25°C cage. However, at the same temperature, the development time of the 21°C flies was shorter than the 25°C flies. This confirmed earlier results of Anderson (1966) for wing length and duration of development. It is usually assumed that development time is positively correlated with body size, so in this respect the results of Anderson (1966) and Lints and Bourgois (1987) are unusual, and they offer no explanation. Lints and

Bourgois (1987) speculate that the extremely rapid genetic divergence observed between their subpopulations could have been due to the action of transposable elements. Their suggestion is in agreement with the work of Rose and Doolittle (1983) and Davidson and Posakony (1982) who proposed transposable elements as important for the phyletic evolution of subpopulations.

Similar temperature-related genetic divergence in wing size has been recorded by Powell (1974) for the tropical species D. willistoni. Most laboratory selection experiments have been done on species of Drosophila from temperate climates where environmental temperature is quite variable. Having a genetic mechanism to cope with temperature change would be selectively advantageous. Tropical species experience less environmental variation in temperature, either seasonally or geographically. Powell (1974) examined two subpopulations of a wild caught stock of D. willistoni, without replicates, established in the laboratory at 25°C and 19°C and examined after 2.5 years. Genetic divergence was measured using the same methods as Anderson (1966) for D. pseudoobscura. Wing length showed genetic divergence, in the same direction as that observed by Anderson (1966); flies kept at 19°C had genetically larger wings than their counterparts at 25°C. Unfortunately, because of the lack of replicates in each thermal environment, genetic drift cannot be ruled out as a cause of the population divergence observed by Powell (1974).

As laboratory evolution experiments do tend to follow the trends observed in the wild, it seems very likely that temperature, or some correlate of temperature, is acting as an important selective force both in the laboratory and the wild.

1.8 Environmental effects of laboratory temperature on Drosophila body size and life history

Together with the genetic component to temperature-related size change in Drosophila mentioned earlier, there is a developmental component, because flies from the same genetic stock are larger if reared at lower temperatures (Alpatov 1929b, 1930). Changes in the wing of D. melanogaster caused by rearing at different growth temperatures were examined by Alpatov (1930), and Robertson (1959a, 1959b), who showed that higher temperature during development resulted in smaller wing sizes. Wing and thorax length were greater at lower growth temperatures in the different geographic populations of D. melanogaster and D. simulans examined by Tantaway and Mallah (1961). They found that wing and thorax length increased with temperature for all stocks. The ratio of wing length to thorax length also increased at the lower temperatures. Phenotypic variance of wing length was higher at both extremes of temperature (10°C and, especially, 30°C). In laboratory populations of D. melanogaster from tropical Africa and from France, flies had greater fresh weights at lower temperatures, regardless of their geographic origin (David and Bocquet 1975a, 1975b). The studies by Alpatov and Robertson revealed that variation in wing size due to growth temperature was a function almost solely of cell size. Work in this thesis shows that the evolutionary response to temperature also occurs mainly by changes in cell size.

The similarity between these genetic and environmental components of clinal variation for body size in Drosophila suggests

that the developmental response is a form of adaptive phenotypic plasticity (Stearns 1989; Kirkpatrick and Lofsvold 1992).

Lower temperature greatly prolongs life in Drosophila (Loeb and Northrop 1917; Alpatov and Pearl 1929; Powsner 1935). Fertility can also be affected by ambient temperature. For instance, Tantaway (1961a, 1961b) found that laboratory temperature affected the rate of egg production in D. pseudoobscura. Daily egg production was greater at higher temperatures, while longevity and total lifetime egg production were greatest at 15°C and lowest at 27°C. Many fitness components have been found to change when populations are reared at different temperatures (Mourad 1965; Kitagawa 1967; David et al. 1983). Productivity (which consists of different traits such as female fecundity, egg to adult viability, male mating ability and male sterility) and fertility (which is a measure of the production of hatching larvae, and may depend on female and male sterilty and on zygotic viability) increased with increasing temperature for D. melanogaster when investigated by Cavicchi et al. (1989). Adult competitive abilities in three related species (D. affinis, D. algonquin and D. athabasca) were tested at 18°C and 25°C by Fogleman and Wallace (1980). D. athabasca had significantly higher competitive ability at 18°C than at 25°C, and its geographic range extends farther north and up to high altitude. D. affinis exhibited its highest competitive ability at the higher temperature, again probably a reflection of its more southerly range and higher frequency in midsummer than at other times of year. The similarities between laboratory experiments carried out at different temperatures and results from field collected flies once again suggests an important role for

temperature, or other temperature-related factors in determining body size and other life history characters in Drosophila.

1.9 Importance of work on thermal biology

Global environmental change will produce long term and widespread increases in environmental temperature, mainly because of a significant increase in the concentration of greenhouse gases. This increase in temperature is likely to result in changes in body size and other life history characteristics in many invertebrate species. It is probable that organisms previously restricted to lower latitudes will invade higher ones. What will be the result of competition between endemic and invading species? Will species adapted to colder environments suffer because of increased temperature and competition from species adapted to warmer environments or will they be able to adapt quickly enough to withstand these changes? Several studies have already documented rapid evolution in relation to latitude (Prevosti et al. 1990). In addition, the presence of size clines in ectotherms challenges our basic understanding of thermal biology. There is at present no theory as to why these clines occur. This thesis attempts to address some of these important questions, by examining how D. melanogaster adapts to culture at changed temperature in the laboratory

1.10 Aims of the thesis

The work reported in this thesis made use of a large population of D. melanogaster that had been subdivided into replicate

populations cultured in different thermal environments (16.5°C and 25°C) for over six years. The aim of this work was to examine a range of morphological characters and life history traits in the replicate populations, under controlled laboratory conditions, to determine whether any genetic divergence had occured between the flies in the two thermal selection regimes. Pre-adult development times (from egg to pupariation and to adult eclosion) were measured, to determine the effect both of environmental temperature and of selection regime on development; the results of these experiments are reported in Chapter 3.

Patterns of larval growth are known to be affected by environmental growth conditions such as food and temperature. Additionally, over six years of thermal selection may have produced genetic differentiation with respect to larval growth rate and the "critical larval weight" at which pupariation is triggered. I set about investigating this issue by recording the weights of precisely aged larvae growing under standard conditions, and by selectively starving individual aged and weighed larvae to determine the critical larval weights at each developmental temperature, and for larvae from each selection regime. The results are reported in Chapter 4.

The ability to compete with conspecifics is an important determinant of success, both for larvae and adults. I examined the competitive ability of larvae from each selection regime at each developmental temperature. Competitive abilities were recorded at low, medium and high larval densities, in competition with mutant marked stocks. The results from these larval competitive ability experiments are given in Chapter 5.

The effects of selection regime and developmental temperature on adult body size were also investigated by measuring thorax length, wing area, cell area and total cell number in the wing. The results of these experiments are presented in Chapter 6.

In Chapter 7, the results of experiments conducted to determine the effect of thermal selection regime and environmental temperature on longevity of adult flies are reported. Finally, I examined female egg-laying ability, together with egg hatchability and total lifetime reproductive success, in females from both selection regimes at both environmental temperatures. The results of these experiments are reported in Chapter 7.

Chapter 2 GENERAL MATERIALS AND METHODS.

2.1 Contents

This chapter describes the food media and the origin and general maintenance of the stocks used in the research. Methods of collection of larvae and virgin adult flies which are common to many chapters are described, to avoid further repetition.

2.2 Food Media

Lewis Medium:

This consisted of 13.75g agar, 187.75g sugar, 207.05g maize meal, 37.05g flaked yeast, 60ml nipagin and 2200ml water.

Grape-juice medium:

This consisted of 40g agar, 500ml water and 300ml grape-juice.

Sugar-Yeast Medium:

This consisted of 30g agar, 100g sugar, 100g yeast, 1000ml water, 30ml nipagin, 3ml propionic acid.

Insect ringer solution:

37.4g of Sodium Chloride (NaCl)

1.75g of Pottasium Chloride (KCl)

1.30g of Calcium Chloride ($CaCl_2$),

0.50g of Di-sodium Hydrogen Phosphate (Na_2HPO_4)

0.25g of Potassium Di-Hydrogen Phosphate (KH_2PO_4).

This was made up to 5l with distilled H_2O.

2.3 The Stocks

The Drosophila melanogaster used to establish the thermal selection lines were derived from a random-bred wild-type stock, collected in June 1984 by Dr. G. Wilkinson in Brighton, England. This stock was then established in Edinburgh and maintained in the laboratory by mass culture in population cages which were housed in a 25°C constant temperature room with a fixed illumination cycle of 12 hours light/12 hours dark. The stock was kept under these conditions until January 1985, when it was subdivided into two thermal groups, one kept at 18°C, the other remaining at 25°C. Each thermal group was subdivided into three replicate cage populations. After one year, the three cages at 18°C were transferred to 16.5°C, and have been maintained at this temperature since. Both temperature regimes are associated with a fixed illumination cycle of 12 hours light and 12 hours dark.

The population cages were maintained using one-third pint glass bottles containing approximately 70 cm^3 of Lewis medium (see section 2.2). Some granular live yeast was added to the surface of the medium. Adult flies fed on yeast and laid eggs upon the Lewis medium in the unstoppered culture bottles in the cages, and larvae developed within the medium. The bottles also served as high humidity refugia for the adult flies. At 25°C, each cage contained four sets of three culture bottles, with a four weekly rotation. At 16.5°C each cage had a total of six sets of three culture bottles with a six weekly rotation. Once per week, the oldest set of bottles were emptied of adults before being removed and replaced by a fresh set of bottles. At both temperatures, the cages contained bottles that had been in the cage for a different length

of time and so contained progeny at different stages of development. The rotation periods at each temperature ensured the complete emergence of all flies in the bottles before they were removed from the cage.

The competitive ability of larvae from the thermal stocks was examined in competition with a mutant-marked stock. A breeding programme was undertaken using balancer chromosomes to generate mutant stocks with chromosomes X, II and III from the 'high' or 'low' thermal stocks (see Figure 2.2 for details). The phenotypic marker used was the eye mutation sparkling-poliert, located on the fourth chromosome. The mutant marker has no discernible effect on larval competitive ability (Lindsley and Grell 1968). Flies homozygous for sparkling-poliert have eyes slightly smaller than normal, with a glassy, shiny appearance and numerous ommatidial hairs and small pits over the surface of the eye. The mutant is easily scored in adults emerging in mixed culture with wild types. (Drosophila Information Service, nº 68, Jan. 1990, part 4: P. 226).

2.4 Collection of Adult Flies and First Instar Larvae for Experiments

The following is a general description of the procedures that were used to collect larvae and flies for experiments. There were some detailed variations to these procedures, described in the relevant chapters.

To obtain larvae and flies for use in experiments, unstoppered yeasted bottles were placed in each of the three cages at both 16.5°C and 25°C. Bottles were left in cages until a

fine sprinkling of eggs had been laid on the surface of the medium. These culture bottles were then emptied of adults, removed from the cage and stoppered. Half of the bottles from each cage were transferred to the other thermal regime, while the other half were kept at the temperature of origin. The flies produced from these bottles were the parents of the experimental flies, and both experienced growth at the same temperature. Four experimental groups were thus set up:

(1) 25°C selected, reared at 25°C.

(2) 16.5°C selected, reared at 25°C.

(3) 25°C selected, reared at 16.5°C.

(4) 16.5°C selected, reared at 16.5°C.

By this procedure, the experimental animals reared at a different temperature from that at which they had been selected, were removed from their selection temperature by one generation. This was done to standardise any effects of the parental environment carrying over to the experimental animals.

Parental flies were collected not less than three hours and not more than eight hours after eclosion, by gently banging each bottle inverted over another fresh culture bottle. Because Drosophila will not mate within eight hours of eclosion at 25°C, all collected flies were virgins. In adult flies less than three hours old, carbon dioxide anaesthesia results in a rapid diffusion of carbon dioxide into the gut, which can rupture it and cause death (David and Hout 1973). After this early sensitive period, short exposure to carbon dioxide does not significantly affect longevity or progeny production of females (Partridge et al. 1986). The parental flies were later sorted under carbon dioxide anaesthesia into small cylindrical translucent plastic containers (65mm x 37mm) with

dishes (see Figure 2.1). Each dish contained approximately 10ml of grape-juice medium, with a small paste blob of brewer's yeast paste on the moist surface of the medium. Females readily laid eggs on the surface of the grape-juice medium. Flies were regularly transferred to new dishes by gently inverting and banging the container, then quickly replacing the old dish with a new and freshly yeasted dish. The purpose of these transfers was to prevent egg-retention by the females, making them produce a steady flow of eggs, all at the same very early stage of development. (In the absence of oviposition sites females often retain maturing eggs in their oviducts, and these hatch early and therefore interfere with the determination of development times and competitive ability.) A penultimate period of egg laying, the 'pre-lay', was followed by a final measured time period, referred to hereafter as the 'lay'. About 49-52 hours after the midpoint of the lay at 16.5°C, and 25-26 hours after the lay midpoint at 25°C, first instar larvae were picked from the dish under the x25 objective of a binocular microscope, using a very fine paintbrush. The first instar larvae were large enough to pick easily off the medium without damaging them. Larvae were placed into small shell vials (75mm x 25mm) which contained 7ml of Lewis medium, with a drop of watery yeast mixture added onto the medium to provide a moist environment for the young larvae. The exact number of larvae placed into each vial depended upon the particular experiment.

When virgin adults were required for experiments, they were collected from larval cultures of the type just described. Before the subjective dawn vials were emptied of any adults. Five hours later any adult flies present were collected by gently

banging each vial in turn over a single fresh vial. After a further three hours, the collected adults were anaesthetised with carbon dioxide gas, and separated as male and female virgins. Therefore, this five hour/three hour procedure ensured that all flies were virgins and were old enough to withstand the short anaesthesia required for sexing and separating.

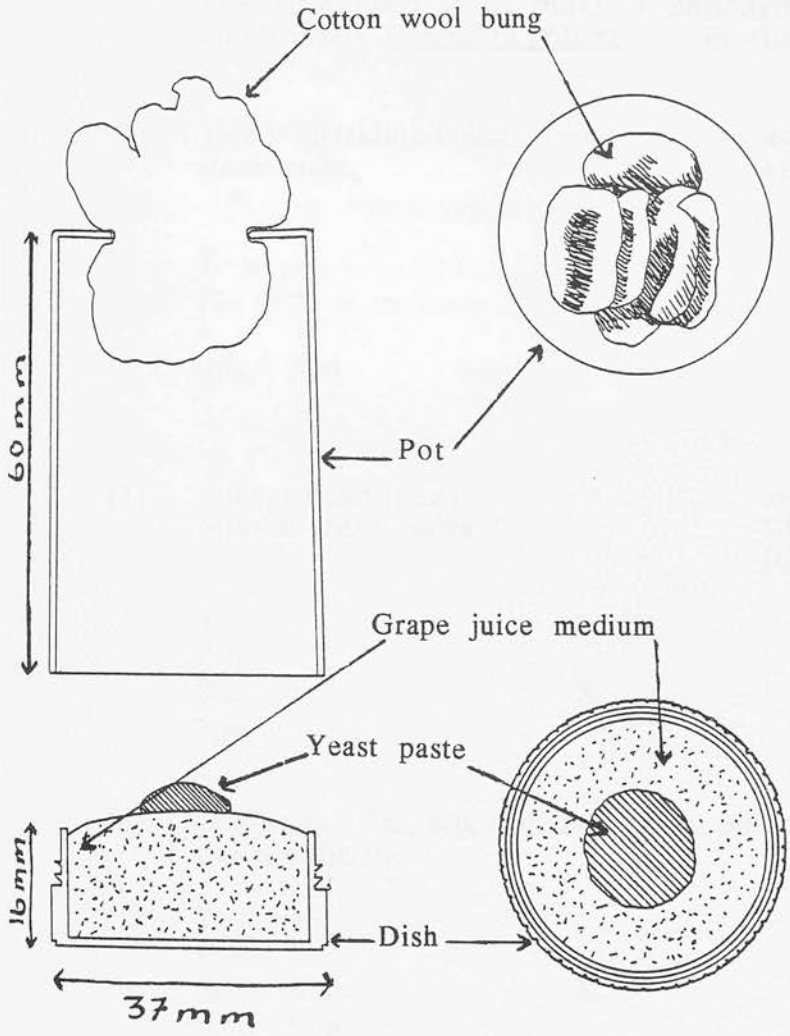


Figure 2.1 Section and Plan of 'grape juice' containers used for the collection of first instar larvae of the same early stage of development. This was used in experiments where development times between selected lines were being contrasted, and larval numbers, maturity and growth conditions needed to be controlled.

Figure 2.2 Details of the balancer program used to generate the Sparkling Poliert stocks used for experiments in Chapter 5.

(A). The first step is to make a 2nd/3rd chromosome balancer stock with Sparkling Poliert on the 4th chromosome.

(1) Take a Sparkling Poliert (Spa) stock male and a II III balancer virgin female.

Y	+		+	+
+	+		Cy	Plm
+	+	X	Stb	Ubx
Spa	Spa		+	+

(2) Collect Plum (Plm), Stubble (Stb) males and Curly (Cy), Ultrabithorax (Ubx) virgin females

Y	+		+	+
Plm	+		Cy	+
Stb	+	X	Ubx	+
Spa.	+		spa.	+

(3) Collect Cy, Plm, Stb, Ubx, Spa males and virgin females to start the stock

Y	+		+	+
Plm	Cy		Plm	Cy
Stb	Ubx	X	Stb	Ubx
Spa	Spa		Spa	Spa

(B). The next step is to introduce the thermally selected stocks into the program.

(4) Take Fm7 males and virgin female II III balanced Spa stock from (3).

Y	Fm7		+	+
+	+		Plm	Cy
+	+	X	Stb	Ubx
+	+		Spa	Spa

(5A) Collect Fm7, Cy, Ubx virgin females

+	Fm7		Y	+
+	Cy		+	+
+	Ubx	X	+	+
+	Spa		+	+

and introduce wild type males from selected line

(5B) Running simultaneously with (5A).

Collect male II III balanced Spa stock from stage (3)

Y	+		+	+
Cy	Plm		+	+
Ubx	Stb	X	+	+
Spa	Spa		+	+

and introduce wild type virgin females from selected line

(6) From (5A) collect Fm7, Cy, Ubx virgin females

+	Fm7		Y	+
+	Cy		Plm	+
+	Ubx	X	Stb	+
+	+		Spa	+
or (+	Spa)			

From (5B) collect Plm, Stb males

(7) Collect wild type with Spa. (i.e. NOT Cy, Plm, Stb or Ubx)

+	+	Y	+
+	+	+	+
+	+	+	+
Spa	Spa	Spa	Spa

(Themally selected line, either 16.5°C or 25°C, with Sparkling poliert on 4th chromosome)

Chapter 3 EFFECT OF THERMAL SELECTION ON LARVAL DEVELOPMENT TIMES

3.1 Introduction

Rate of development is an important life history variable (Charlesworth 1980). Drosophila melanogaster may often undergo periods of population expansion during which early breeding is favoured. These periods are interspersed with episodes of rapid, density-independent population decline during which life history variables are irrelevant. Under these circumstances rapid development is favoured by natural selection (Lewontin 1965). In addition, slow larval development is associated with higher larval mortality (Partridge and Fowler 1993), again favouring rapid development.

The development rate from egg to adult emergence of insects in general, and Drosophila in particular, has been repeatedly shown to have a temperature coefficient, similar to the temperature dependence of many chemical reactions (Loeb and Northrop 1917; Pittendrigh 1954; Ohnishi 1976; Lints 1978; McKenzie 1978). The dependence of average duration of development of male and female D. melanogaster on developmental temperature was found to have a biphasic form by Economos and Lints (1984a, b, c; 1986a, b). The duration of development decreased quickly with temperatures between 12°C and 21°C and then more slowly towards a minimum value at about 38°C. Above this temperature, the duration of

development increased with temperature, presumably because the upper lethal limit was approached.

The relationship between developmental temperature and the duration of different stages of the developmental period in D. melanogaster was studied by Powsner (1935). With decreasing developmental temperature, the length of the embryonic period increased, as did the larval period. Female larval periods were found to be longer, and affected more by decreasing temperature, than male larval periods. The length of pupal periods again showed an increase with decreasing developmental temperature, but in this case male pupal periods were greater and affected more by temperature than were those of females. In general, females eclose earlier than males (Sokoloff 1955; Miller 1964; Tantaway and Soliman 1965; Fogleman and Wallace 1980). The developmental time (egg to adult) in D. mercatorum has also been shown to decrease with increasing developmental temperature (Ikeda and Saito 1983).

Considerable variations in development times have been reported in many natural populations of Drosophila, reared under standard laboratory conditions (e.g. D. pseudoobscura, Dobzhansky et al. 1942). In general, development times decrease with increasing latitude and increasing altitude of collection, as mentioned in Chapter 1.

It has been demonstrated that the rate of development in Drosophila can also evolve in response to long term thermal selection in the laboratory. Duration of development of D. melanogaster was measured at regular intervals over a period of six years in populations kept at 21°C, 25°C and 28°C (Lints and Bourgois 1987). All flies were tested under standard

conditions at 25°C and at 28°C. When tested at 25°C, the flies from the 21°C cages were larger, but had shorter development times than the flies from the other cages. This confirmed similar results of Anderson (1966) for the evolution of development time in D. pseudoobscura (see Introduction). In other contexts, long development time is usually associated with larger body size (e.g. Alpatov 1929b, 1930; Partridge and Fowler 1993), so in this respect these results are somewhat curious.

In an independent study, using the same populations as described and used in this thesis, Huey et al. (1991) measured development time (to eclosion) at 16.5°C and at 25°C, for flies from thermal selection regimes of 16.5°C and 25°C. Development times of flies from both selection regimes were roughly twice as long at low temperature as at high temperature. The effect of rearing temperature differed between selection regimes, with "high temperature" flies developing faster than "low temperature" flies at 25°C, but the "low temperature" flies developing faster than "high temperature" flies at 16.5°C. Huey et al. (1991) also recorded significant heterogeneity between the replicate lines within each selection regime. These results indicate that the thermal dependence of development time had evolved during the course of "natural selection" in the laboratory.

The work reported in this chapter repeats and extends the previous work of Huey et al. (1991), who measured only time to eclosion. In this study, time to pupariation, the duration of the pupal period and also total time to eclosion were measured under standard conditions for both high and low temperature flies, at each developmental temperature.

3.2 Time to pupariation and eclosion at low density

3.2.1 Materials and methods

The aim of this experiment was to compare the development times of the selected lines from the 16.5°C and 25°C selection regimes at both developmental temperatures. Times to pupariation and to eclosion were determined.

Two egg-collections were made from each set of three cages at 16.5°C and 25°C; eggs from one collection were reared at 16.5°C, and from the other at 25°C. Development to eclosion took 9-10 days at 25°C and 23-24 days at 16.5°C. In order to have synchrony in eclosion, eggs for development at 16.5°C were collected 12-13 days before those for 25°C development. The parental flies and the experimental larvae therefore developed at the same experimental temperature.

Freshly eclosed parental flies were collected by gently tapping each culture bottle inverted over a fresh, lightly yeasted food bottle. After ageing for several days, the parental flies were sorted under carbon dioxide anaesthesia and transferred to containers with grape juice medium at a density of 50 males and 50 females per container.

Flies were maintained in grape juice containers overnight, transferred to new containers for two hours, and then transferred to new lids for the lay, which lasted for 2 hours at 25°C and 6 hours at 16.5°C. Early first instar larvae were picked from the surface of the medium 25-26 hours after the midpoint of the lay at 25°C and 49-52 hours after the midpoint of the lay at 16.5°C.

Twenty vials of yeasted Lewis medium, each with 32 larvae

were set up from each of the replicate lines for development at each temperature. Times to pupariation and to eclosion were measured. Before the third instar larva is ready to pupariate it usually leaves the food and ascends the side of the glass vial. It will often wander around before eventually becoming sluggish. Eversion of the anterior spiracles soon follows, and the larva then takes on a more rotund appearance and subsequently darkens. It was by the appearance of the everted anterior spiracles that pupariation was scored. Each vial was checked every 8 hours at 25°C and every 12 hours at 16.5°C, and the number of pupae present was recorded. The number of emerged adults was also recorded every 8 hours/12 hours, by gently tapping out the flies over a single fresh food vial. These flies were later counted and sexed under carbon dioxide anaesthesia.

3.2.2 Results

Figures 3.2.1(a) and (b) show the mean times to pupariation in hours from mid point of lay, of larvae from both selection regimes, reared at both 16.5°C and 25°C. (See also Appendix A; Table A1). An analysis of variance (with the three cage lines nested within each of the two selection regimes) was used to examine the effects of growth temperature, selection temperature, replicate cage line within selection regime, and of their interactions on the time to pupariation. The results of this analysis are shown in Table 3.2.1

The effect of growth temperature on time to pupariation was clear; this was much longer at 16.5°C than at 25°C. The main effect of selection regime was also significant, with larvae from

the 25°C selection regime having longer pupariation times at both 25°C and 16.5°C. There was also considerable heterogeneity between the replicate cage lines, nested within the selection regimes. The growth temperature by selection regime interaction was not significant, indicating that there was no difference in the extent to which larvae from the two selection regimes responded to the two growth temperatures.

The mean development times to eclosion at both developmental temperatures, for males of both selection regimes, are given in Figures 3.2.2(a) and (b). The corresponding data for females are presented in Figures 3.2.3(a) and (b). This data is also given in Appendix A, Tables A2 and A3. The results of the analysis of variance, as described previously, are given in Tables 3.2.2 and 3.2.3. There was again a clear difference due to growth temperature, development times to eclosion being about twice as long at 16.5°C as at 25°C for both sexes. The three replicate cage lines of each selection regime again showed considerable heterogeneity. The main effect of selection regime was not significant for either males or females. There was a significant interaction between growth and selection temperatures for both sexes. At 25°C, the 16.5°C selected males and females developed relatively slowly, eclosing around 3-4 hours after their counterparts selected at 25°C. The situation was reversed at 16.5°C, with 25°C selected males and females developing relatively slowly compared with 16.5°C selected lines.

The pupal period was calculated for each vial by subtracting the mean pupariation time from the mean eclosion times of males and females. Figures 3.2.4(a) and (b) and Figures 3.2.5(a) and (b) give the mean pupal periods with 95% confidence limits for lines

of each selection regime at each developmental temperatures, for males and females respectively. (See Appendix A; Tables A4 and A5). The results of the nested analyses of variance are given in Tables 3.2.4 and 3.2.5. There was a clear difference due to growth temperature, with pupal periods being about twice as long at 16.5°C as at 25°C for both sexes. There was no significant main effect of selection in females. Males from the 16.5°C selection regime had slightly longer pupal periods at both 25°C and 16.5°C, as shown by the significant main effect of selection. Both sexes showed a considerable heterogeneity between cage lines within each selection regime. There was a significant interaction between growth and selection temperatures for both sexes, indicating that there was a difference in the extent to which pupae from the two selection regimes responded to the two growth temperatures. One can see from Figures 3.2.4(a) and (b) that in males the differences in pupal period between the selection regimes are greater at 25°C than at 16.5°C. In females the direction of the difference in pupal period between the selection regimes reversed, with 25°C lines having shorter pupal periods at 25°C and 16.5°C lines having shorter pupal periods at 16.5°C.

TABLE 3.2.1 NESTED ANALYSIS OF VARIANCE
Times to pupariation

source	ss	df	ms	F	P
growth temperature	1525394	1	1525394	24603.129	***
selection temperature	1188	1	1188	16.971	**
lines within selection[A]	279	4	70	8.75	***
growth x selection	388	1	388	5.543	N.S.
growth x lines within[B]	246	4	62	7.75	***
error[C]	1862	288	8		

* = P < 0.05 ** = P < 0.01 *** = P < 0.001

A.....Lines within selection is the error term for selection temperature.

B.....Growth x lines within selection is the error term for both growth temperature and for growth x selection.

C.....Error is the error term for lines within selection and growth x lines within.

TABLE 3.2.2 NESTED ANALYSIS OF VARIANCE
Times to eclosion for males.

source	ss	df	ms	F	P
growth	5052904	1	5052904	61620.78	***
selection	96	1	96	0.419	N.S.
lines within selection[A]	918	4	229	17.615	***
growth x selection	2021	1	2021	24.646	**
growth x lines within[B]	328	4	82	6.308	***
error[C]	3017	228	13		

* = P < 0.05 ** = P < 0.01 *** = P < 0.001

Error terms as in Table 3.2.1

TABLE 3.2.3 NESTED ANALYSIS OF VARIANCE
Times to eclosion for females.

source	ss	df	ms	F	P
growth	4946762	1	4946762	20027.377	***
selection	1359	1	1359	4.356	N.S.
lines within selection[A]	1250	4	312	19.5	***
growth x selection	2805	1	2805	11.356	*
growth x lines within[B]	988	4	247	15.438	***
error[C]	3705	228	16		

* = P < 0.05 ** = P < 0.01 *** = P < 0.001

Error terms as in Table 3.2.1

TABLE 3.2.4 NESTED ANALYSIS OF VARIANCE
Pupal period for males.

source	ss	df	ms	F	P
growth	1020355.227	1	1020355.227	18565.415	***
selection	791.304	1	791.304	14.123	*
lines within selection[A]	224.110	4	56.028	3.931	**
growth x selection	770.596	1	770.596	14.021	*
growth x lines within[B]	219.840	4	54.960	3.856	**
error[C]	3249.866	228	14.254		

* = P < 0.05 ** = P < 0.01 *** = P < 0.001

Error terms as in Table 3.2.1

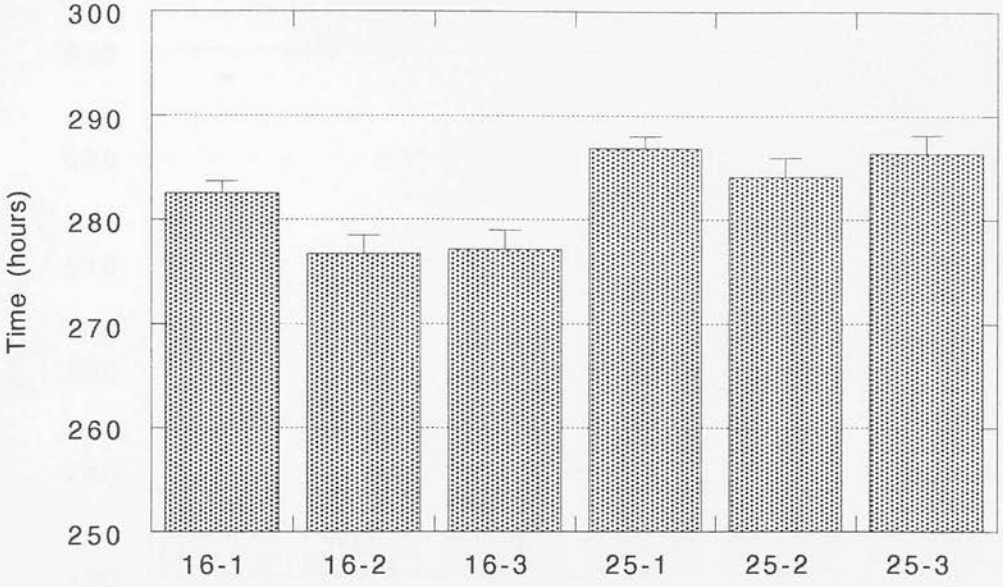
TABLE 3.2.5 NESTED ANALYSIS OF VARIANCE
Pupal period for females.

source	ss	df	ms	F	P
growth	968098.173	1	968098.173	7368.183	***
selection	8.415	1	8.415	0.1374	N.S.
lines within selection[A]	244.973	4	61.243	5.168	***
growth x selection	1473.419	1	1473.419	11.214	*
growth x lines within[B]	525.558	4	131.389	11.087	***
error[C]	2701.976	228	11.851		

* = P < 0.05 ** = P < 0.01 *** = P < 0.001

Error terms as in Table 3.2.1

Figure 3.2.1(a) Mean pupariation times at 16.5°C
(+/- 95% confidence intervals)



(b) Mean pupariation times at 25°C
(+/- 95% confidence intervals)

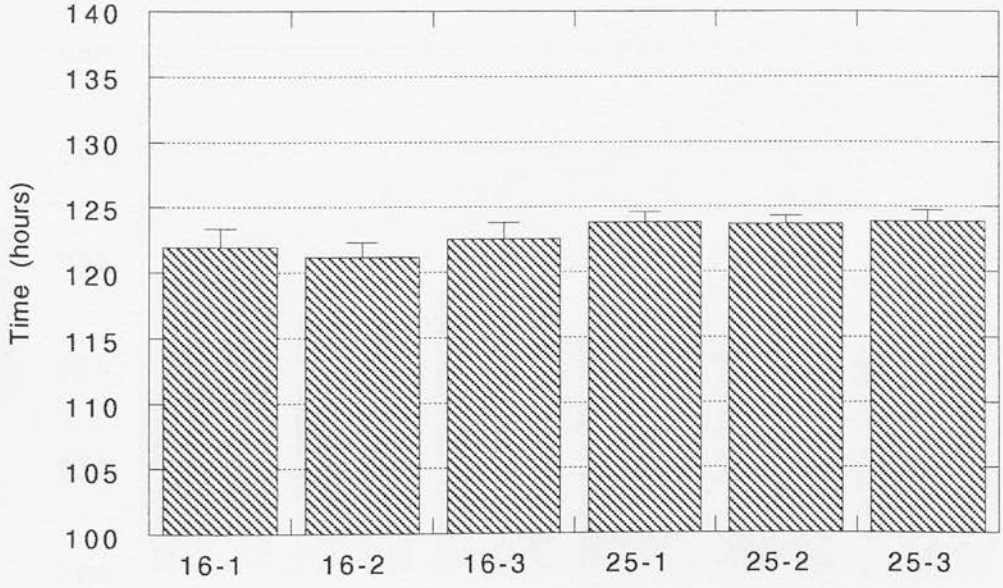
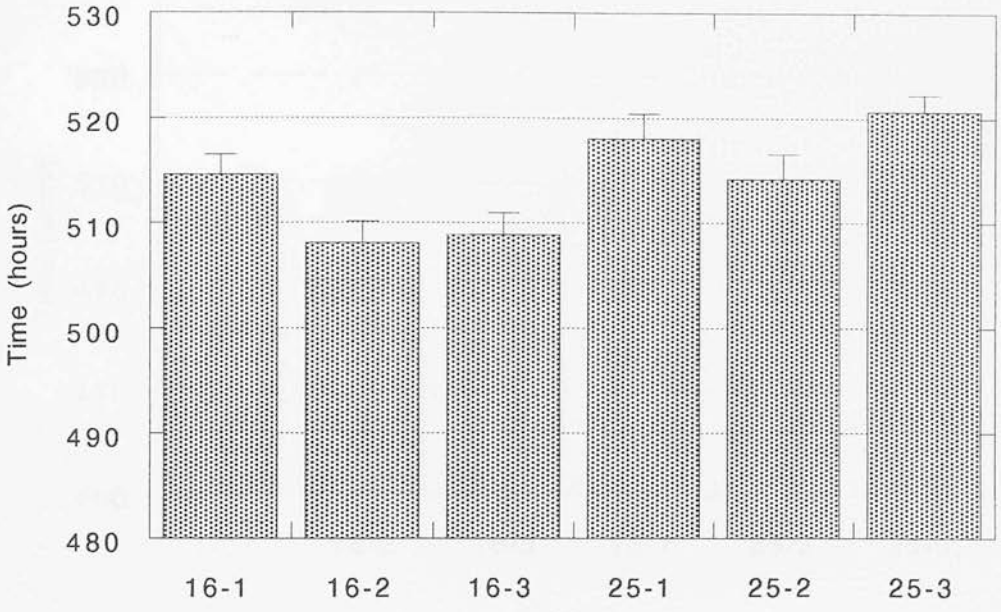


Figure 3.2.2(a) Mean eclosion times for males at 16.5^{0}C
(+/- 95% confidence intervals)



(b) Eclosion times for males at 25^{0}C
(+/- 95% confidence intevals)

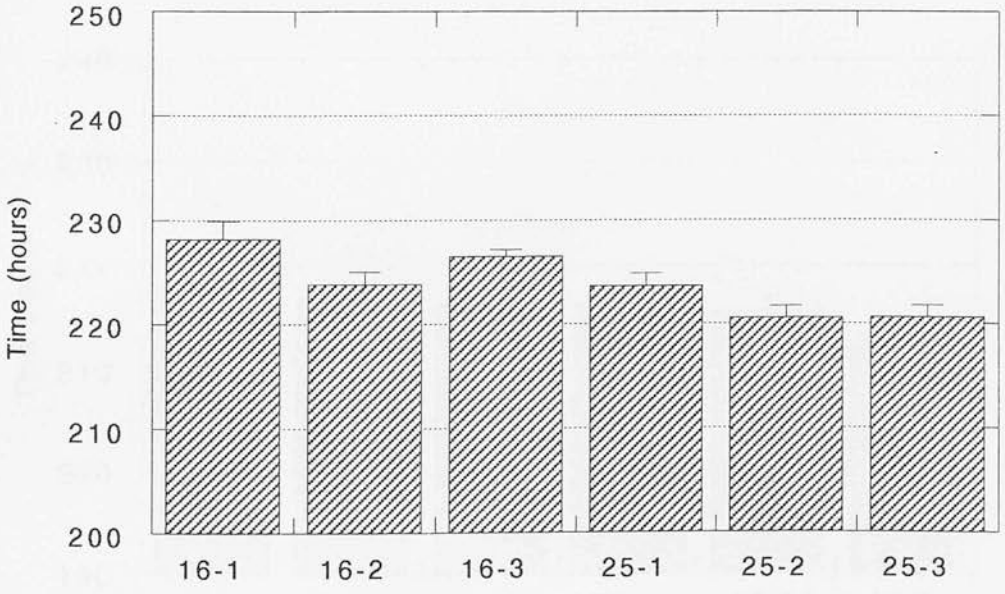
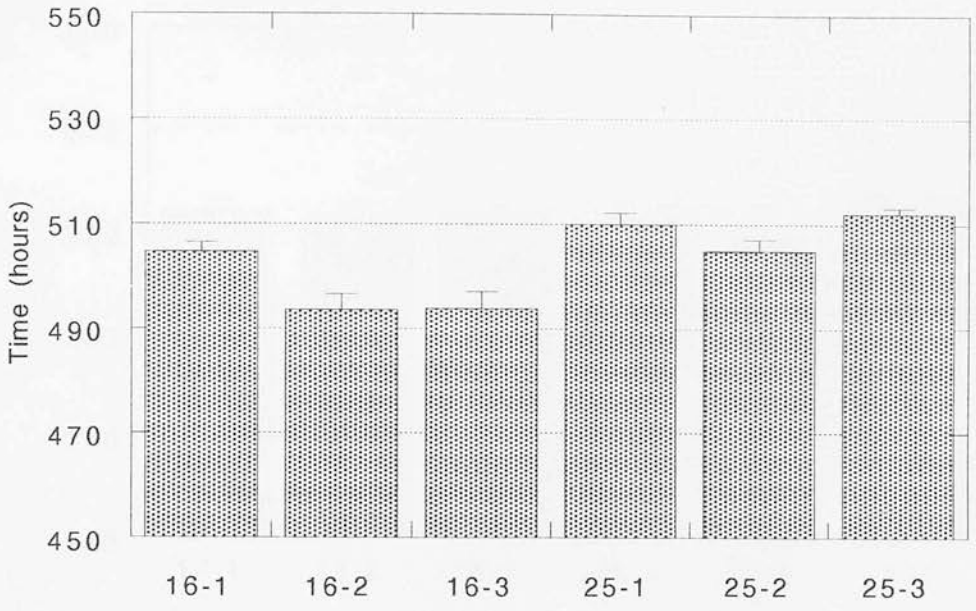


Figure 3.2.3(a) Mean eclosion times for females at 16.5°C
(+/- 95% confidence intervals)



(b) Mean eclosion times for females at 25°C
(+/- 95% confidence intervals)

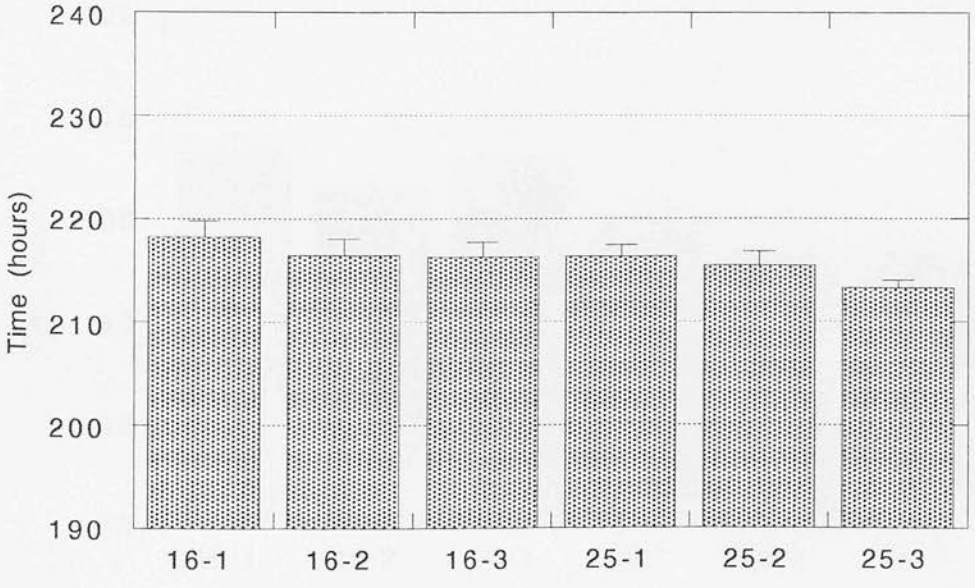
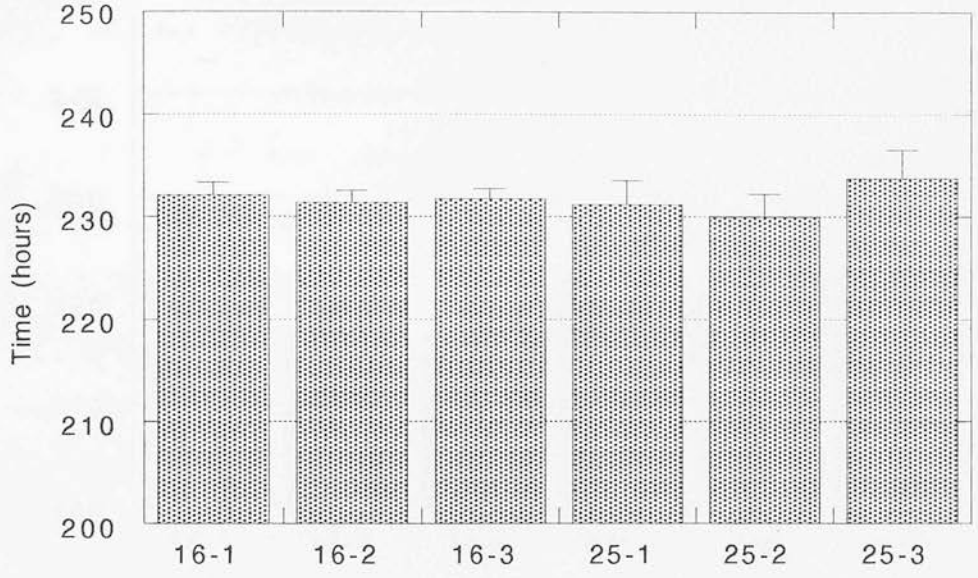


Figure 3.2.4(a) Mean pupal period for males at 16.5°C
(+/- 95% confidence intervals)



(b) Mean pupal period for males at 25°C
(+/- 95% confidence intervals)

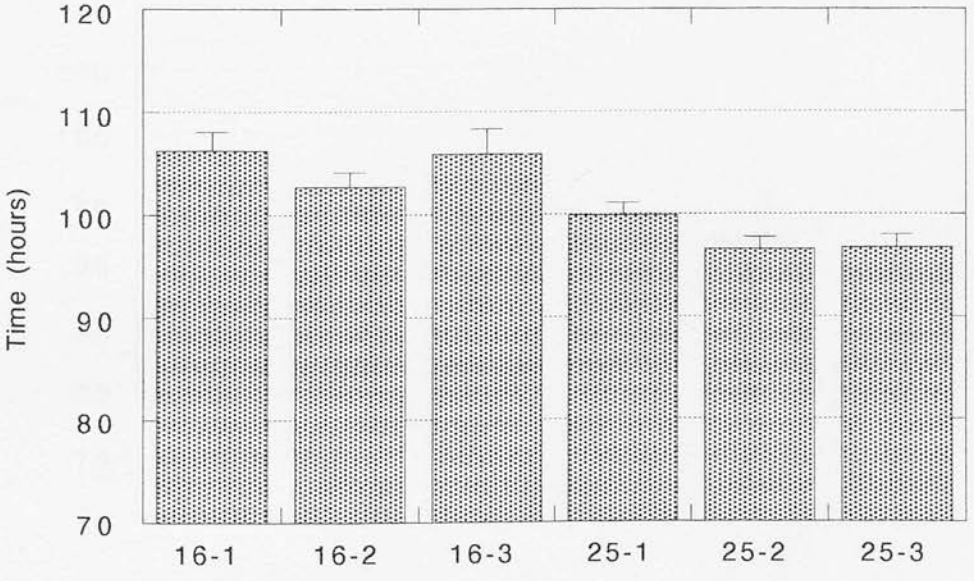
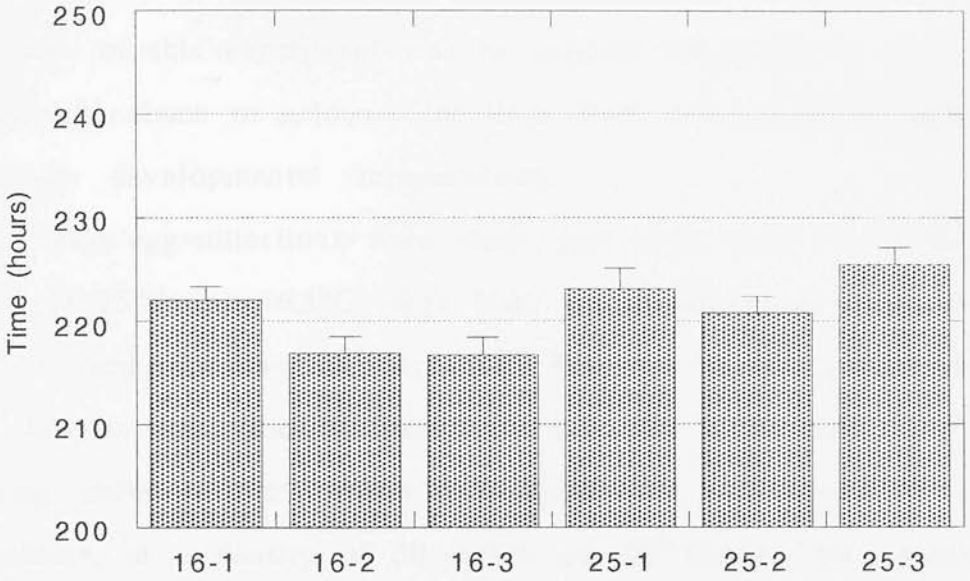
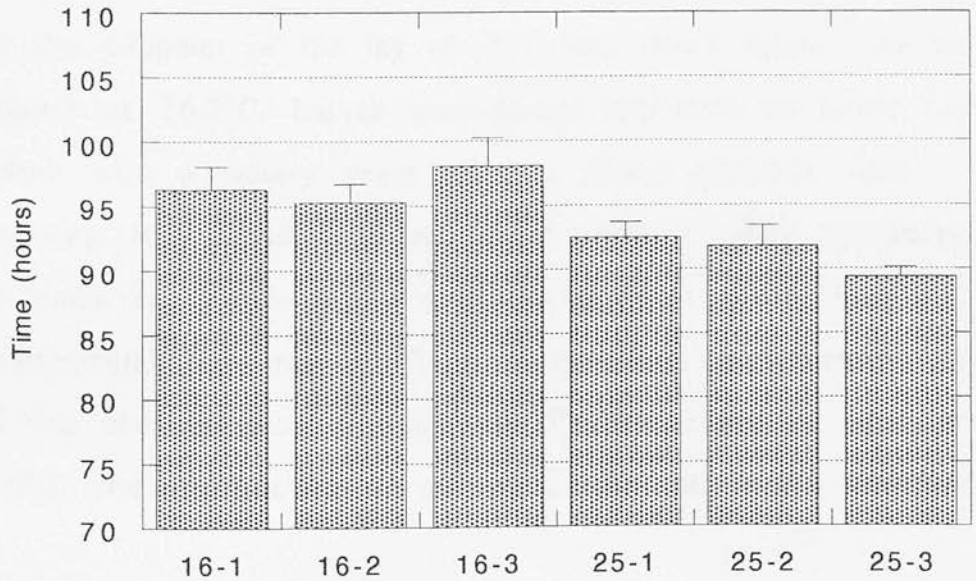


Figure 3.2.5(a) Mean pupal period for females at 16.5^{0}C
(+/- 95% confidence intervals)



(b) Mean pupal period for females at 25^{0}C
(+/- 95% confidence intervals)



3.3 Development times in relation to larval density

3.3.1 Materials and Methods

The aim of this experiment was to examine the effect of larval density on times to eclosion for lines from both selection regimes at both developmental temperatures.

Two egg-collections were made, one from each set of three cages at 25°C and 16.5°C; eggs from one collection were reared at 16.5°C, and from the other at 25°C. Parental flies were collected and held in fresh food bottles for some days to mature, before sorting under carbon dioxide anaesthesia into grape juice containers, at a density of 50 males and 50 females per container. The flies were maintained in these containers for 24 hours and then transferred to fresh containers for a further 12 hours. Two short 3 hour pre-lays then followed, and the flies were finally transferred to fresh containers for the lay, which lasted for 6 hours at 25°C and 12 hours at 16.5°C.

First instar larvae were picked from the lids 24-26 hours after the midpoint of the lay at 25°C and 48-51 hours after the midpoint at 16.5°C. Larvae were placed into vials on Lewis food medium with a watery yeast mixture. Three replicate vials containing 100 larvae each and three replicate vials containing 600 larvae each were set up for each replicate line at each developmental temperature. Time to eclosion was measured. Each vial was checked every 8 hours at 25°C and every 12 hours at 16.5°C, and the number of newly emerged adults was recorded.

3.3.2 Results

All figures have corresponding Tables, these can be found in Appendix A. The mean times to eclosion for males and females at larval densities of 100 per vial at both developmental temperatures are given in Figures 3.3.1(a) and (b) and 3.3.2(a) and (b). The results of the analyses of variance on these data are given in Tables 3.3.1 and 3.3.2.

The development times in both sexes were much longer at the lower temperature. There was a significant effect of selection regime for females, but not for males. The effect of growth temperature on development time differed between the selection regimes, as shown by the significant interaction term. 16.5°C selected lines developed relatively slowly at 25°C, whereas at 16.5°C it was the 25°C selected lines which had a longer time to eclosion. Males showed significant heterogeneity between cage lines within each selection regime, but no such significant variation was found between replicate cage lines for females.

Development times at larval densities of 600 per vial are shown in Figures 3.3.3(a) and (b) for males and 3.3.4(a) and (b) for females. There was a considerable increase in times to eclosion at the higher larval density, across all the groups. The results of the analyses of variance are given in Tables 3.3.3 and 3.3.4. The effect of growth temperature on development time was evident for both males and females, with longer times to eclosion at 16.5°C compared to 25°C. There was no significant main effect of selection regime for either sex. There was a significant interaction effect of growth and selection temperature for males. At 16.5°C, the 25°C selected males developed relatively slowly, whereas at

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25°C the 16.5°C selected males developed more slowly. For females at larval densities of 600 per vial this interaction was marginally non significant ($P< 0.10$).

Figures 3.3.5(a) and (b) give the number of adults which emerged from each vial with initial larval density of 100 at each development temperature. This was transformed using the angular transformation, for subsequent nested analysis of variance, the results of which are shown in Table 3.3.5. Neither growth nor selection temperature had a significant effect on pre-adult survival, but there was a significant growth temperature X selection regime interaction. From Figure 3.3.5(b) it can be seen that at 25°C the 25°C selected lines had a higher number of adults emerging than did the 16.5°C selected lines, while at 16.5°C this situation was reversed, as seen from Figure 3.3.5(a). The number of adults emerging at the higher larval density of 600 per vial are given in Figures 3.3.6(a) and (b), and the results of the analysis of variance in Table 3.3.6. At this high density none of the main effects or interactions were significant.

TABLE 3.3.1 NESTED ANALYSIS OF VARIANCE.
Times to eclosion for males at larval density = 100.

source	ss	df	ms	F	P
growth	1184687	1	1184687	25979.978	***
selection	184.05	1	184.05	5.434	N.S
lines within selection[A]	135.49	4	33.87	2.922	*
growth x selection	1034.69	1	1034.69	22.691	**
growth x lines within[B]	182.44	4	45.60	3.934	*
error[C]	278.19	24	11.59		

* = P < 0.05 ** = P < 0.01 *** = P < 0.001
Error terms as in Table 3.2.1

TABLE 3.3.2 NESTED ANALYSIS OF VARIANCE.
Times to eclosion for females at larval density =100.

source	ss	df	ms	F	P
growth	111457	1	111457	18241.735	***
selection	121	1	121	17.460	*
lines within selection[A]	27.73	4	6.93	1.246	N.S.
growth x selection	695.2	1	695.2	113.780	***
growth x lines within[B]	24.44	4	6.11	1.099	N.S.
error[C]	133.54	24	5.56		

* = P < 0.05 ** = P < 0.01 *** = P < 0.001
Error terms as in Table 3.2.1

TABLE 3.3.3 NESTED ANALYSIS OF VARIANCE.
Times to eclosion for males at larval density = 600.

source	ss	df	ms	F	P
growth	1611842	1	1611842	224803.63	***
selection	0.780	1	0.780	0.01936	N.S.
lines within selection[A]	161.112	4	40.28	2.518	N.S.
growth x selection	272.8	1	272.8	38.047	**
growth x lines within[B]	28.67	4	7.17	0.448	N.S.
error[C]	384.919	24	16		

* = P < 0.05 ** = P < 0.01 *** = P < 0.001
Error terms as in Table 3.2.1

TABLE 3.3.4 NESTED ANALYSIS OF VARIANCE.
Times to eclosion for females at larval density = 600.

source	ss	df	ms	F	P
growth	1589280	1	1589280	63826.506	***
selection	10.24	1	10.24	0.2168	N.S.
lines within selection[A]	188.9	4	47.23	4.435	**
growth x selection	149.65	1	149.65	6.010	N.S.
growth x lines within[B]	99.69	4	24.9	2.338	N.S.
error[C]	225.62	24	10.65		

* = P < 0.05 ** = P < 0.01 *** = P < 0.001
Error terms as in Table 3.2.1

TABLE 3.3.5 NESTED ANALYSIS OF VARIANCE.
Pre-adult viability for density = 100 per vial.

source	ss	df	ms	F	P
growth	78.23	1	78.23	6.971	N.S.
selection	0.412	1	0.412	0.0156	N.S.
lines within selection^{A}	105.8	4	26.46	0.6152	N.S.
growth x selection	488.48	1	488.48	43.525	**
growth x lines within^{B}	44.89	4	11.223	0.2609	N.S.
error^{C}	1032.28	24	43.01		

* = P < 0.05 ** = P < 0.01 *** = P < 0.001

Error terms as in Table 3.2.1

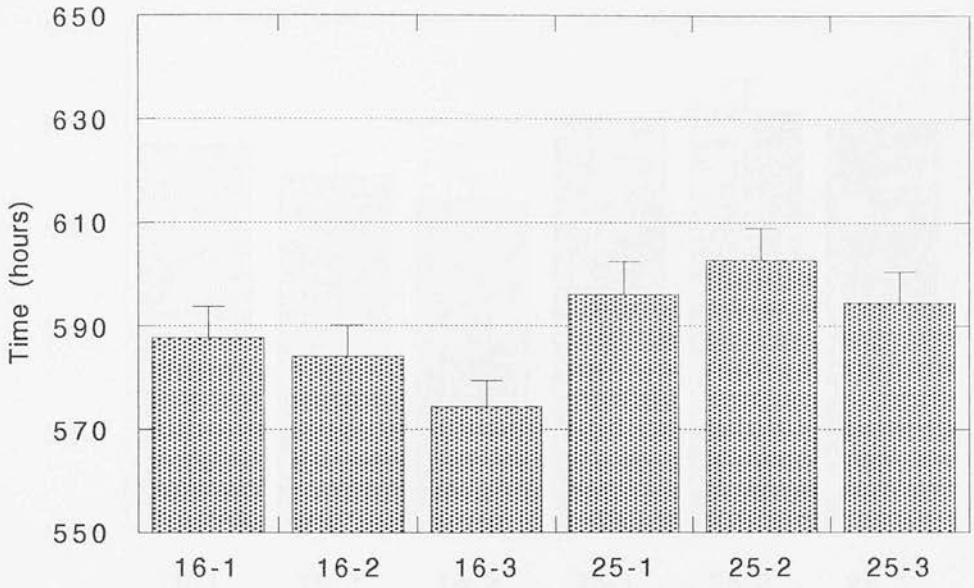
TABLE 3.3.6 NESTED ANALYSIS OF VARIANCE.
Pre-adult viability for density = 600 per vial.

source	ss	df	ms	F	P
growth	31.7	1	31.7	1.909	N.S.
selection	1.03	1	1.03	0.42	N.S.
lines within selection^{A}	101.96	4	24.49	1.90	N.S.
growth x selection	68.17	1	68.17	4.11	N.S.
growth x lines within^{B}	66.65	4	16.6	1.29	N.S.
error^{C}	309.9	24	12.91		

* = P < 0.05 ** = P < 0.01 *** = P < 0.001

Error terms as in Table 3.2.1

Figure 3.3.1(a) Mean eclosion time for males at 16.5°C
Larval density = 100 per vial
(+/- 95% confidence intervals)



(b) Mean eclosion time for males at 25°C
Larval density = 100 per vial
(+/- 95% confidence intervals)

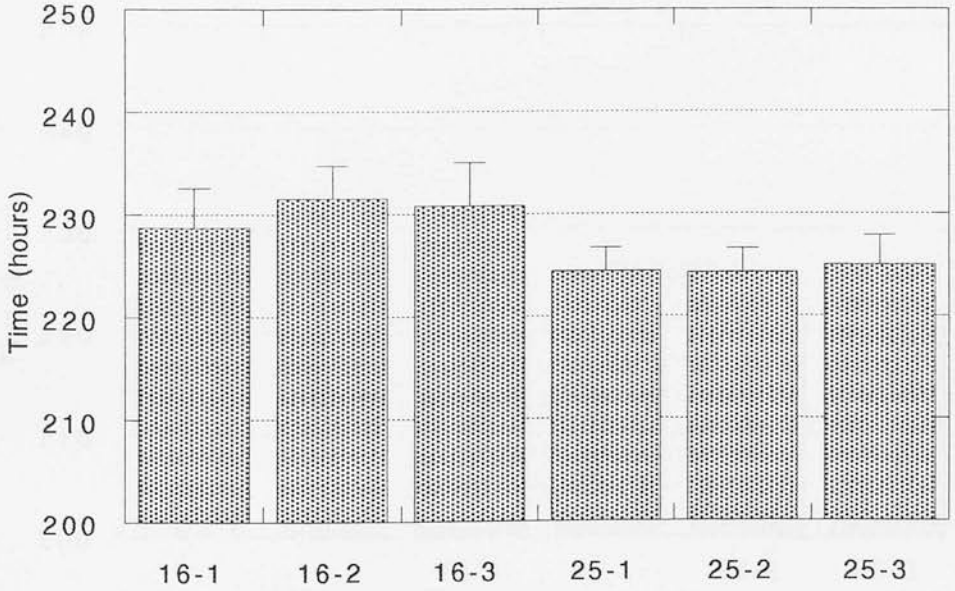
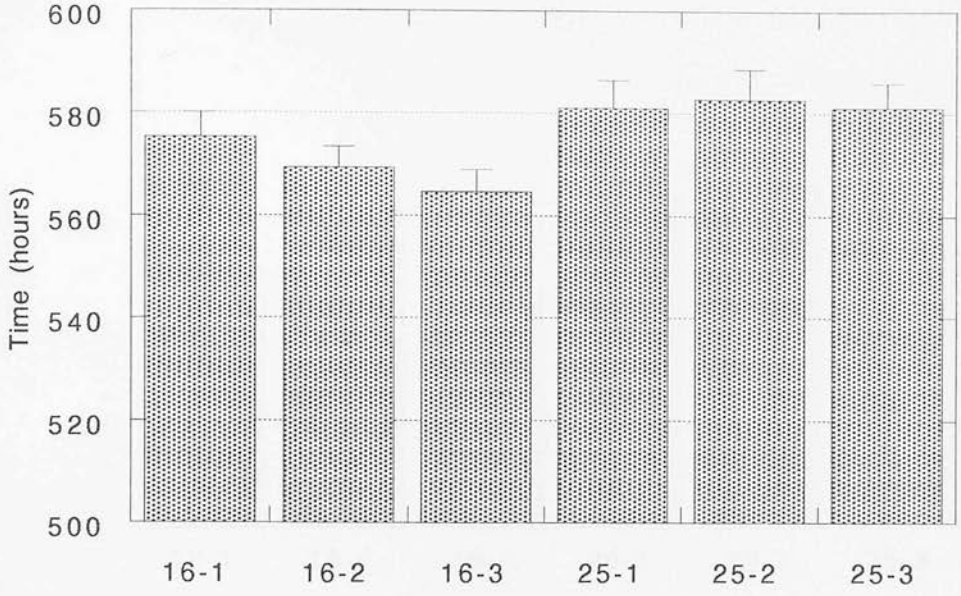


Figure 3.3.2(a) Mean eclosion time for females at 16.5^{0}C
Larval density = 100 per vial
(+/- 95% confidence intervals)



(b) Meaneclosion time for females at 25^{0}C
Larval density = 100 per vial
(+/- 95% confidence intervals)

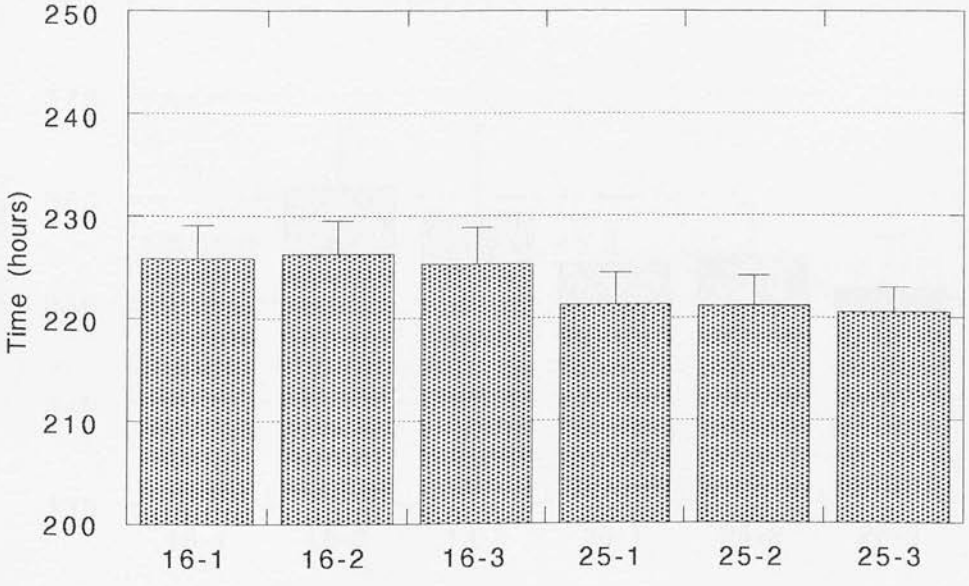
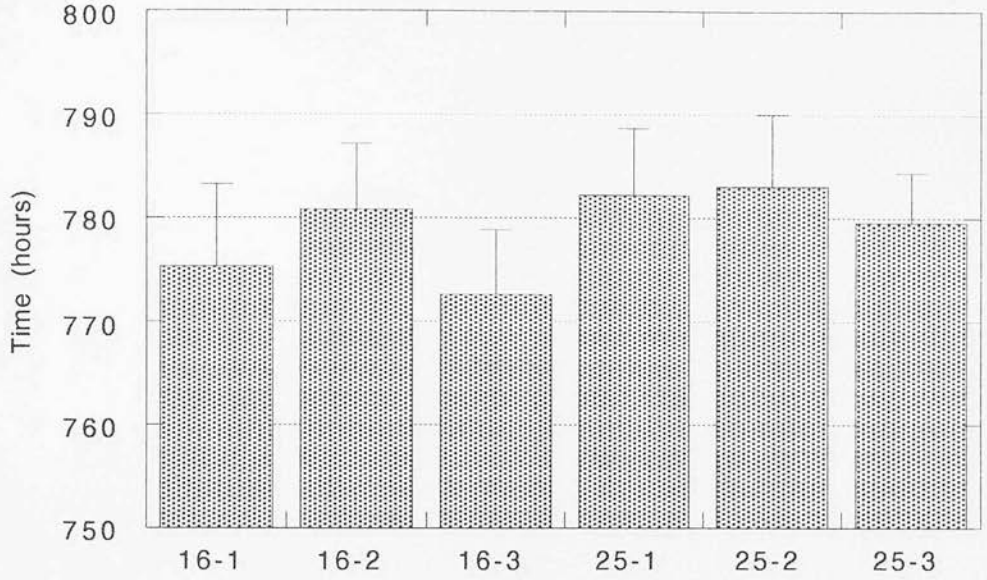


Figure 3.3.3(a) Mean time to eclosion for males at 16.5^{0}C
Larval density = 600 per vial
(+/- 95% confidence interval)



(b) Mean eclosion time for males at 25^{0}C
Larval density = 600 per vial
(+/- 95% confidence intervals)

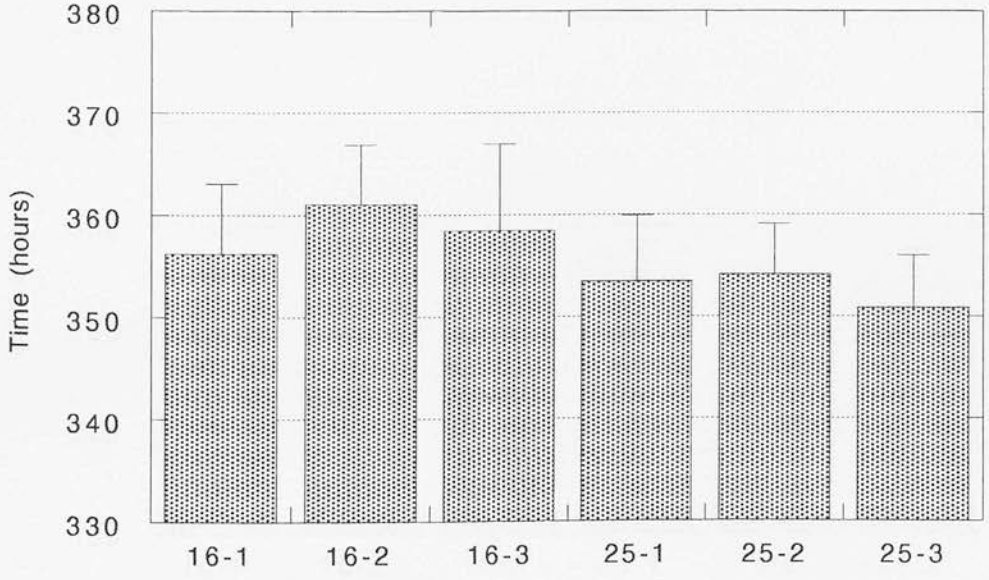
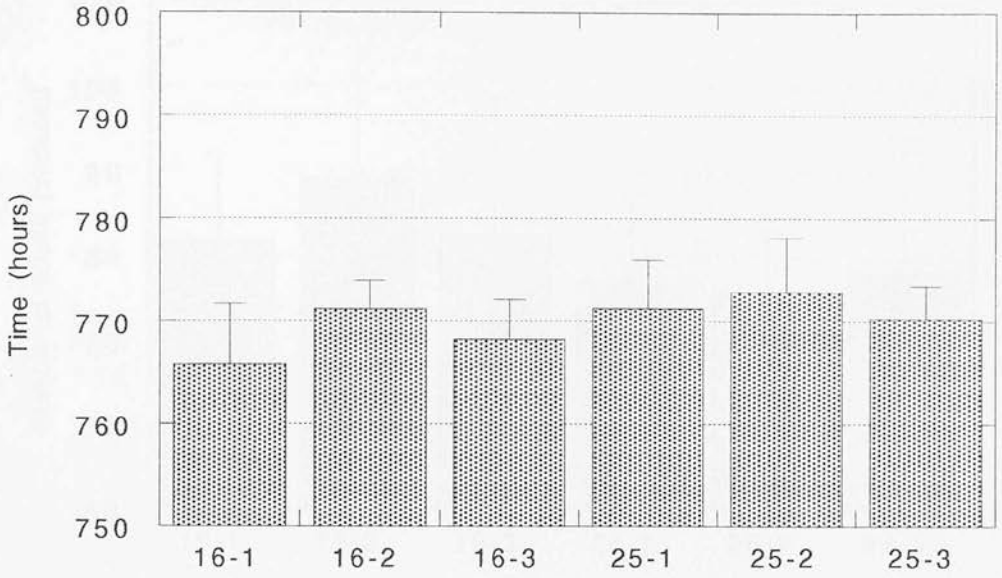


Figure 3.3.4(a) Mean eclosion times for females at 16.5^{0}C
Larval density = 600 per vial
(+/- 95% confidence intervals)



(b) Mean times to eclosion for females at 25^{0}C
Larval density = 600 per vial
(+/- 95% confidence intervals)

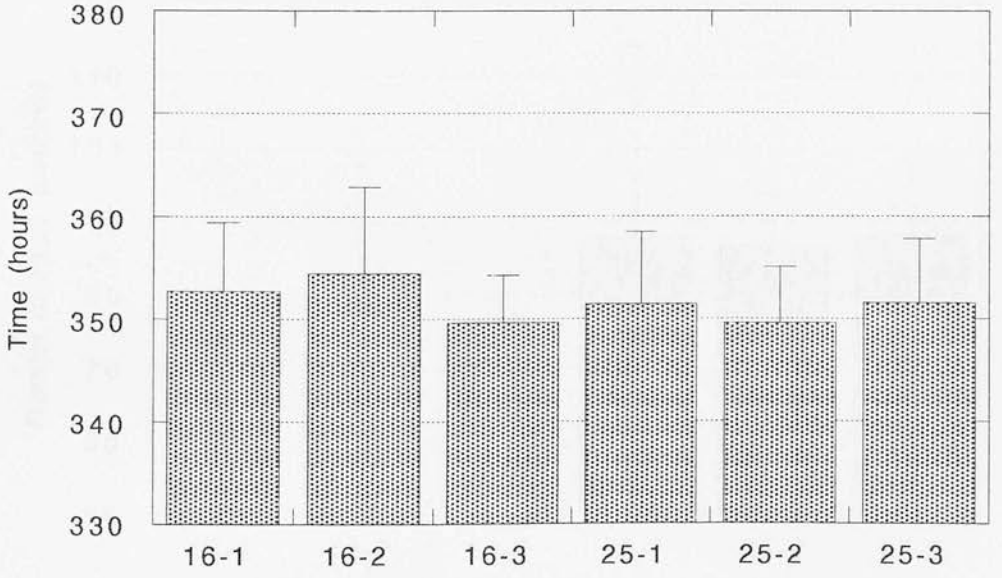
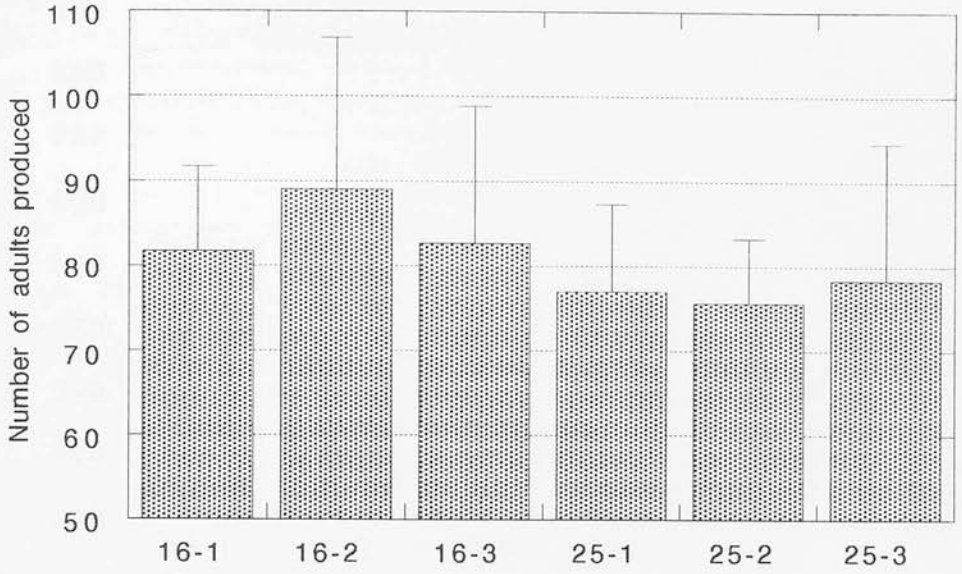


Figure 3.3.5(a) Mean number of adults produced from vials at 16.5°
Larval density = 100 per vial
(+/- 95% confidence intervals)



(b) Mean number of adults produced from vials at 25°C
Larval density = 100 per vial
(+/- 95% confidence intervals)

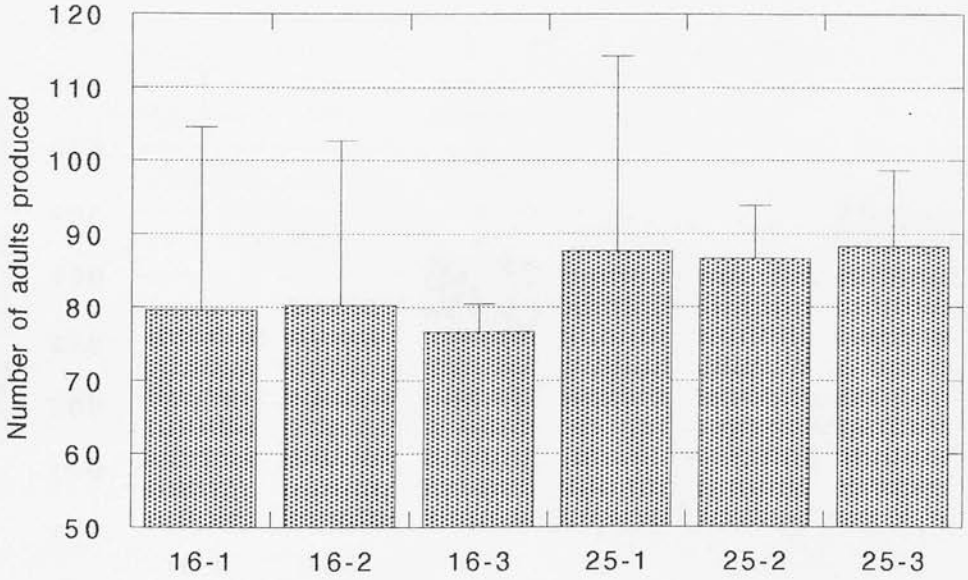
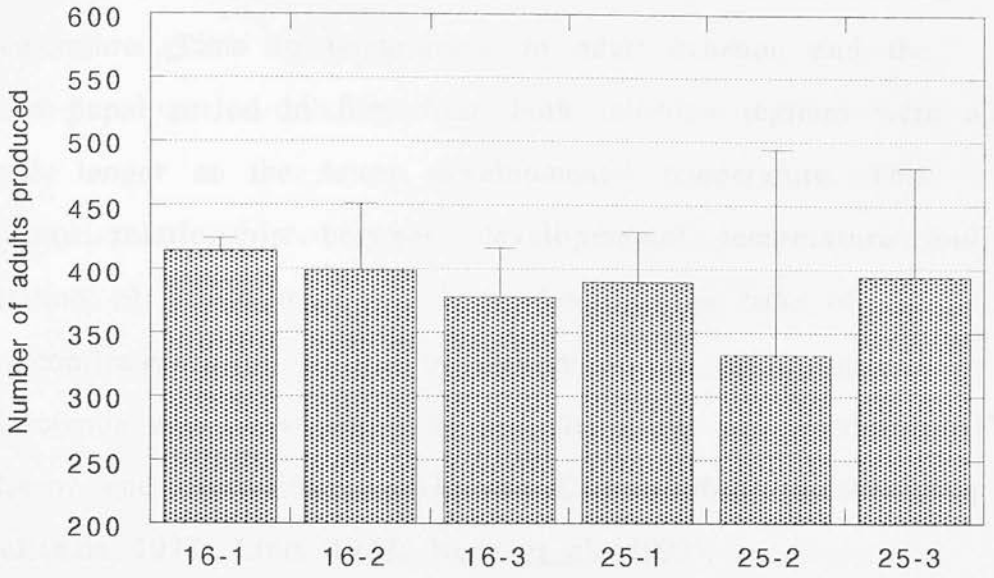
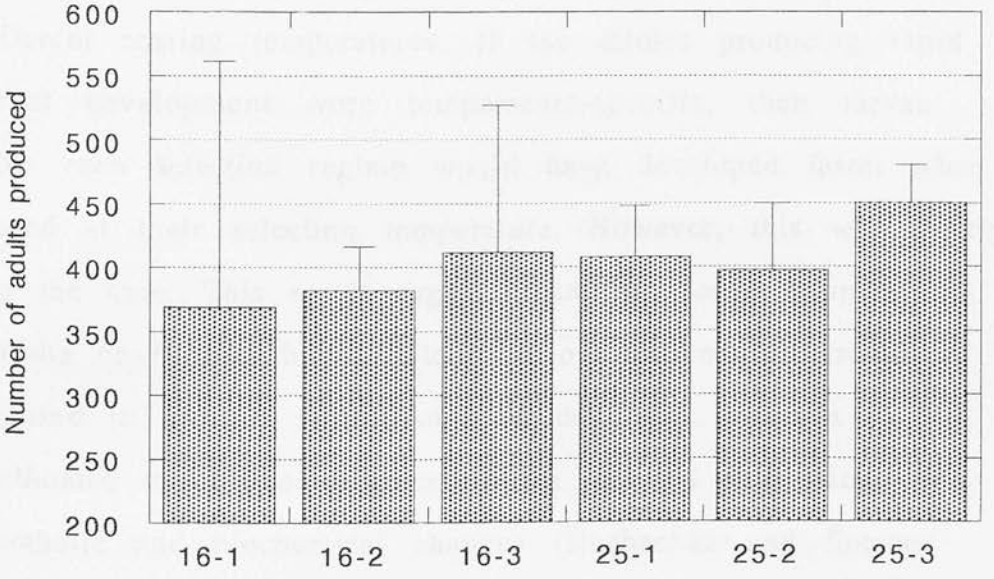


Figure 3.3.6(a) Mean number of adults produced from vials at 16.5°C
Larval density = 600 per vial
(+/- 95% confidence intervals)



(b) Mean number of adults produced from vials at 25°C
Larval density = 600 per vial
(+/- 95% confidence intervals)



3.4 Discussion

The average duration of developmental stages in both male and female D. melanogaster clearly depends on rearing temperature. Time to pupariation, to adult eclosion and the mean pupal period in flies from both selection regimes were all much longer at the lower developmental temperature. This inverse relationship between developmental temperature and duration of development has been described as "one of the overconfirmed facts" of the physiology of D. melanogaster (Economos and Lints 1986a, b; see also Loeb and Northrop 1917; Alpatov and Pearl 1929; David and Clavel 1967; Ohnishi 1976; McKenzie 1978; Lints 1978; Huey et al. 1991).

Times to pupariation of 25°C selected larvae were longer than those of 16.5°C selected larvae, when reared at 25°C or at 16.5°C. Unlike other life history characters, for instance, fertility (see Chapter 7), development time of larvae did not show any gene x environment interaction with temperature. The direction of the difference between selection regimes stayed the same at different rearing temperatures. If the alleles producing rapid larval development were temperature-specific, then larvae from each selection regime would have developed faster when reared at their selection temperature. However, this was clearly not the case. This result suggests that the lower temperature permits or favours higher allocation of resources to growth as opposed to somatic maintenance of the larva. Animals acclimated to cold environments often show a wide range of metabolic and biochemical changes (Hochachka and Somero 1984; Pain 1987), such as enzymes with higher turnover rates

and lower Q_{10} values. This would compensate for the reduced thermal energy encountered by these animals (Hochachka and Somero 1973; 1984). Thermal selection may favour the evolution of enzymes that can function in cold environments via enhanced enzyme ligand affinity at low temperatures and reduced subunit bonding energies, both of which would permit normal allosteric behaviour at low temperatures (Heinrich 1981; Hazel 1984; Bowler and Fuller 1987; Cossins and Raynard 1987). It could be that the shorter pupariation times of the 16.5°C selected larvae at both growth temperatures reflected a higher enzyme activity/affinity, due to adaptation to the colder environment. However, why the development rate of the 16.5°C selected larvae was not higher only at the lower growth temperature is puzzling.

Times to adult eclosion at 16.5°C were shorter for the 16.5°C selected lines than for the 25°C selected lines. However, at 25°C the situation was reversed, with 16.5°C selected lines developing relatively slowly compared to 25°C selected lines. There were similar results when time to eclosion was measured at higher larval densities of 100 and 600 larvae per vial.

The present results of development time to adult eclosion are similar to those of other thermal evolution studies in D. melanogaster (Lints and Bourgois 1987) and results reported by Huey et al. (1991) using the same populations of D. melanogaster used in these experiments.

Separation of the pre-adult period into larval and pupal periods revealed that males and females from the 16.5°C selected lines had longer pupal periods at 25°C than did 25°C selected animals. At 16.5°C, males from the 16.5°C selected

lines had significantly longer pupal periods than 25°C selected males. However, 16.5°C selected females had shorter pupal periods compared to 25°C selected females at 16.5°C. It could be that the longer pupal period of the 16.5°C selected lines at 25°C is a direct result of their rapid pre-pupal development. The 25°C selected lines had a greater time for additional larval growth, and possibly accumulated more resources to complete pupal development at a faster rate than 16.5°C selected lines. Alternatively, adult structures in 16.5°C selected animals may be more costly, or take longer to produce than the adult structures in 25°C selected animals.

Drosophila development rate is also inversely related to larval density (Barker 1973; Ohnishi 1976). Low larval density increases both developmental rate and pre-adult survival (Boulétreau-Merle 1988). These findings are also evident from experiments reported in this Chapter, where development time increased as larval density was increased from 32 larvae per vial up to 100 and 600 larvae per vial. At low larval density, almost all larvae completed development successfully, while at the higher larval densities a smaller percentage of larvae developed successfully (see Figures 3.3.5 and 3.3.6). Higher larval densities are known to increase competition, leading to greater pre-adult mortality and longer developmental periods (Ohnishi et al. 1976; Scheiring et al. 1984; Boulétreau-Merle 1988). Increasing larval densities produce greater amounts of toxic biotic residues, such as uric acid and urea, which are believed to increase pre-adult mortality rates (Budnick and Brncic 1974, 1975; Botella et al. 1985). Increased larval density also affects adult characteristics, with body size and weight

being reduced with increased larval density, due to direct competition for food (Lints and Lints 1969, 1971; Economos and Lints 1984b).

In vials set up with 100 larvae, 25°C selected lines had greater pre-adult survival at 25°C than did animals from the 16.5°C selected lines. The situation was reversed at 16.5°C, with the 25°C selected animals having lower pre-adult survival than 16.5°C selected animals. Since pre-adult survival is higher in the lines reared at their own selection temperature, this clearly shows adaptation of the selected lines to their respective thermal environments.

Development time has responded rapidly to thermal selection, with animals having faster development times (to eclosion) and greater pre-adult survival in the thermal environment in which they were selected. As mentioned previously, developmental rate is a key component of fitness (Charlesworth 1980). The observed divergence between the selection regimes in development times presumably reflects the action of natural selection on these lines. The similarity in results between the replicate lines of each selection regime strongly suggests that the differentiation between the populations from each selection regime is not due to other factors such as random genetic drift. However, as pointed out by Huey et al. (1991), whether this observed divergence reflects evolution in only one or in both selection regimes remains an open question, because the previous thermal history of the lines is unknown. Measurements on the lines when they were founded would have been pointless, because uncontrolled environmental variation will affect measures of life history

traits made in different generations. One way to overcome this problem is to freeze a sample of the initial starting population (Lenski et al. 1991; Bennett et al. 1992), which although it may work for bacteria such as E. coli, is not yet possible with D. melanogaster (Ashburner 1989).

The observed response to thermal selection was very rapid, occurring within the 5 years since the lines were set up. Similar rapid genetic divergence with respect to temperature has been reported in D. pseudoobscura (Anderson 1966, 1973), D. subobscura (Prevosti et al. 1990) and D. melanogaster (Cavicchi et al. 1985). Rapidity of response to natural selection may also be a common feature of evolutionary change, as seen in nature (Gibbs and Grant 1987; Malhotra and Thorpe 1991; Pimm 1988; Prevosti et al. 1990).

Chapter 4 LARVAL GROWTH CURVES AND CRITICAL LARVAL WEIGHTS

4.1 Introduction

The aim of this Chapter was to look at larval growth rates and critical weights for pupariation, because these are both likely to be important in determining (a) larval competitive ability and (b) adult body size, the subjects of Chapters 5 and 6 respectively.

Two important decision points during development are known to affect timing of pupariation and eventual adult size in Drosophila. The first of these crucial decision points occurs at the beginning of the third larval instar (Simpson and Schneiderman 1975, 1976). If larvae are removed from the food medium before the early third instar and starved of food, they can survive beyond the normal pupariation time, but they ultimately fail to pupariate (Beadle et al. 1938). Larvae that are removed from the food medium after the early third instar pupariate on time, but as small larvae, which produce small adults (Beadle et al. 1938; Robertson 1963). Controlled feeding experiments have shown that variation in the level of nutrition before the early third instar affects both pupariation time and eventual adult size. However, variation in nutrition after the early third instar affects only adult size; the pupariation time remains unchanged (Robertson 1963). These results suggest that a critical larval weight, necessary for pupariation to occur, is achieved at the beginning of the third larval instar. Work on other insects (mainly Hemiptera and Lepidoptera) has also

demonstrated a critical weight or size which potentiates larval molting (Nijhout 1981). This critical point is followed by a set period during which the level of feeding will largely determine the final adult size (Robertson 1963; Simpson et al. 1980).

Imaginal discs are the precursors of many adult structures in holometabolous insects such as Drosophila. The imaginal appear to discs have a final target cell number. This is controlled by a mechanism intrinsic to the disc itself and it is not directly linked to the endocrine signals that determine the end of the larval period (Bryant and Simpson 1984; Steel and Davey 1985; Zdarek 1985; Bryant 1987). The intrinsic nature of this mechanism is shown by experiments in which the imaginal discs are provided with extra time for cell proliferation. These experiments involve one of two basic procedures: discs are either removed and cultured in young larval or adult hosts, or some discs are selectively damaged by the application of temperature pulses to mosaic larvae bearing tissue with a temperature sensitive cell-lethal mutation. This latter procedure retards pupariation and provides the wild type imaginal discs with an extra period of time for growth before the start of metamorphosis. In both these cases, it would appear that the discs do not increase in cell number or volume or produce larger body structures by extra growth, instead they produce the normal final cell number and normal sized adult structures, although this has not yet been rigorously demonstrated to be the case. (Simpson and Schneiderman 1976; Simpson et al. 1980; Adler 1981; Bryant and Levinson 1985).

Larval maturation is not regulated to coincide with imaginal development and maturation. However, proliferating

imaginal cells can exert a negative influence on the timing of pupariation (Poodry and Woods 1990). It is known that pupariation can occur in the complete absence of imaginal discs (Poodry 1975; Simpson et al. 1980; Szabad and Bryant 1982). Poodry and Woods (1990) reported the results of experiments designed to examine the contributions of larval and imaginal cells to the timing of pupariation. A high dose of gamma-irradiation (10Krad) was used to selectively delete the imaginal cells from the developing larvae of D. melanogaster. Those animals depleted of imaginal cells pupariated when the larval cells were allowed to mature. The conclusion from this was that imaginal discs were unnecessary for pupariation to occur and that larval cells contained the 'primary developmental timer' for pupariation (Poodry and Woods 1990).

Experiments reported in Chapter 6 show that 16.5°C selected lines have larger body and wing size than 25°C selected lines, when reared at either of the growth temperatures. To achieve a greater size, the 16.5°C lines would have to either extend their developmental period or alter their larval growth rate. However, in lines selected for large size, larval growth rate did not increase (Robertson 1963), suggesting that increased target size may be achieved through extension of the growth period alone. Longer larval development times could reduce fitness and increase cumulative larval mortality, as already mentioned in Chapter 3. However, larval development times of 16.5°C selected lines were shorter than those of 25°C selected lines at both growth temperatures (Chapter 3). Increased adult body size could be achieved by an increase in critical weight, as suggested by Partridge and Fowler (1993).

The experiments described in this chapter were designed to measure growth rate and to examine the effects of larval age and weight upon the initiation of pupariation. Synchronously hatched larvae were reared under controlled ad libitum feeding conditions, and weighed to determine larval growth curves at each developmental temperature for both sexes from each selection regime. To determine critical weights for pupariation, larvae were starved at known age and weight, to determine whether or not they pupariated.

4.2 Materials and Methods

In the first set of experiments larval growth curves were determined. To obtain larvae from both thermal selection regimes, reared at both developmental temperatures, parents of the experimental larvae were obtained in the standard way, described in Chapter 3.2, and eggs collected. Early first instar larvae were picked from the grape juice laying dishes and placed on to clear agar growth dishes (made with 12g of agar dissolved in 1000 ml of distilled water) with several drops of yeast paste. The larvae were allowed to develop on these clear agar dishes, and fed regularly with fresh yeast paste. Several drops of distilled water were also added to the agar dishes every day, to prevent dehydration of the larvae. Larvae were picked from three lays A, B and C, staggered in time by several hours, giving three groups of larvae at different stages of development at any one time. Larvae from lays A and B were used in the determination of the larval growth curves, while

larvae from lays B and C were used fore the larval starvation experiment (see later).

At each sampling point, 50 larvae from each line of each selection regime were picked from a growth dish, placed in a small metal sieve, and washed with insect Ringer solution (see Chapter 2 for details) to remove any yeast or other debris. Each larva was weighed on a Sartorius M500p micro balance to the nearest 0.002mg and placed individually in a small glass vial (12mm x51mm) containing a strip of tissue paper soaked with insect Ringer and a small drop of yeast paste. This provided a moist environment and food for the larva to develop, so that its sex could be determined in adulthood. Larval weights were obtained every 12 hours at 25°C and every 24 hours at 16.5°C.

The growth curves were calculated for all cage lines, but critical larval weights for pupariation were determined for a single replicate line from each thermal selection regime at each growth temperature. Parental flies from all replicate lines were examined to determine those most likely to yield sufficient larvae for the experiment. The two lines chosen were 25-1 and 16-2. For the determination of critical larval weights two separate lays were used, using the same replicate lines in each lay. This therefore enabled two starvation experiments to run in tandem at each growth temperature. Larvae were picked from a growth dish and weighed individually. Larvae were removed from food every eight hours between 184 hours and 224 hours from the mid-point of the lay at 16.5°C, and every four hours between 72 hours and 96 hours from the mid-point of the lay at 25°C. Fifty larvae were picked from a growth dish, washed and then weighed individually. These larvae were placed in the

small glass vials with tissue paper soaked in insect Ringer, but no yeast paste was added i.e. the larvae were starved. The vials were stoppered and later examined for adults, dead larvae or pupae. If the larva had reached its critical weight when it was placed in the vial it would pupariate successfully and emerge as an adult. If it had not yet reached its critical weight, since there was no food in the vial, it would not pupariate successfully. This starvation experiment therefore enabled the critical larval weight to be determined. Larvae that had been placed in marked vials with insect Ringer and yeast were later sexed as adults, or, if they failed to eclose, they were sexed in the pupa if possible.

4.3 Results

The mean larval weights (+/- 95% confidence limits) at each time interval, for males that developed at 25°C, are given in Figure 4.1. Figure 4.2 presents similar information for female larvae at 25°C. At each sampling point the selected lines were handled as pairs. Therefore, at each time interval for both male and female growth curves, the data were analysed as matched pairs, and the probabilities from these independent tests of significance were combined using the method outlined by Sokal & Rohlf 1981: pp 779-782). The results of these analyses are given in Tables 4.1- 4.4.

Larvae collected at 26 and 36 hours were not sexed, but the 16.5°C and 25°C selected lines nevertheless showed significant divergence even at that early stage. Larvae from the 16.5°C selected line had significantly greater weights (see Table

4.1). Larvae from both selection regimes showed rapid growth from 60 hours onwards. The selection regimes became distinctly separated at 72 hours and 84 hours, with 16.5°C selected larvae still heavier. A peak weight was reached by larvae from both selection regimes around 96 hours followed by a plateau and slight decrease in weights at 108 hours.

There were significant differences in female larval weight from 48 hours onwards, with the exception of 72 hours. Females selected at 16.5°C were significantly heavier throughout most of the experiment.

Figure 4.3 shows the larval growth curves for male larvae of both selection regimes, reared at 16.5°C, and Tables 4.3 and 4.4 give the results of the statistical analysis. The length of the larval growth period more than doubled for all larvae at 16.5°C. Larvae from both selection regimes reached a much higher weight than did larvae reared at 25°C. Larvae from the selection regimes showed significant differences in weight from 55 hours onwards, but not at 120 hours. From Figure 4.3 one can see a sharp divergence in weight between the selection regimes from 192 hours onwards, with the low temperature larvae having the greater mean weights at each time interval. From Figure 4.3, the difference between the selection regimes at 16.5°C appeared more distinct and persisted for proportionately longer than at 25°C. The pattern for female larvae at 16.5°C followed that for males as can be seen in Figure 4.4, and in Tables 4.3 and 4.4. Again, the low temperature larvae had higher mean weights at almost all time intervals, with the exception of 72 hours, and this pattern appeared more distinct at 16.5°C than at 25°C. Both male and female larvae from the

16.5°C selection regime appeared to have higher weights than the 25°C selected larvae, at almost all time intervals measured.

Figure 4.5 shows the mean larval weights (+/- 95% confidence limits) for the two lays of each selection regime in the 16.5°C starvation experiment. Figure 4.6 gives the results for the corresponding experiment at 25°C. As for the growth curve experiment, the 16.5°C selected line was heavier at each sampling point at each growth temperature. The relationship between weight and age therefore differed between the two lines, with the 16.5°C selected line having greater weights for their age compared to 25°C selected lines. Figures 4.5 and 4.6 also show large differences and variability in mean larval weights between the two lays of a single selection line.

Inspection of the data showed that in the 16.5°C experiment the smallest larvae capable of giving rise to adults weighed between 0.4 and 0.5mg (Figure 4.7) while in the 25°C experiment larvae weighing less than 0.7 - 0.8mg did not produce adults (Figure 4.8). As can be seen from Figures 4.7 & 4.8 there was no obvious difference between the 16.5°C and 25°C experiments in the weight at the start of the plateau for production of adults (approx. 80% and 1.1mg) in relation to larval weight at starvation.

Data for each experiment were divided into larval weight and age categories, because the data suggested that both larval weight and larval age influenced the likelihood of pupariation occuring and an adult being produced. Examination of the data suggests that for a given weight the younger cohort pupates more often at 16.5°C and that for a given age, the heavier larvae pupate more often at 25°C and 16.5°C. Within each

weight and age category, the number of larvae not producing and producing adults at both growth temperatures were recorded, together with the percentage giving rise to adults. This information is presented in Tables 4.5 - 4.8. Within each experiment, every category for which both the 25°C and 16.5°C lines had at least one larva present were compared for the proportion of larvae giving rise to adults. For the 16.5°C experiment, in 23 out of the 30 comparable cells examined, the 25°C selected line gave rise to a higher proportion of adults. Of the remainder, 3 resulted in a tie and in 4 the 16.5°C selected line gave a higher proportion of adults (Sign test p=0.002). At 25°C 32 cells were compared, with the 25°C selected line producing more adults in 6 of these. There were ties in 7 cells and in the remaining 19 the 16.5°C selected line gave rise to a higher proportion of adults (Sign test=0.044). Therefore, in the 25°C experiment the 16.5°C selected lines had significantly lower critical weights, while in the 16.5°C experiment the 25°C selected lines had the significantly lower critical weights for pupariation. Repeating the above analysis and ignoring any cells with less than 5 animals produces the same result, and if anything the result is stronger. However, one problem with this analysis which may urge caution in the interpretation of results, is that larval weights within each selected line at each time interval varied considerably between the two lays, as inspection of Figures 4.5 and 4.6 indicate.

4.4 Discussion

When Drosophila are reared at low growth temperatures the length of time taken for larval growth increases dramatically for all groups of flies, as can be seen from Figures 4.1 - 4.4. This is similar to the previously described effect of growth temperature on development times (Chapter 3). In the present experiment, the larval period more than doubled for both selection regimes when they were reared at 16.5°C compared to 25°C. Furthermore, larvae from both selection regimes reached a higher weight when reared at 16.5°C compared to 25°C.

The experiment reported in this chapter is the first examination of the growth curves and critical weights of larvae which have undergone long term thermal selection. An evolutionary response of larval growth to the two selection temperatures was apparent. The 16.5°C selected line had higher mean weights at almost all sampling intervals at both growth temperatures, in comparison to the 25°C selected line.

As has been mentioned previously (section 3.4), animals acclimated to cold environments often show specific metabolic and biochemical changes such as higher enzyme reaction rates and efficiencies which may tend to compensate for the reduced thermal energy in the environment. This could be the case in the present experiment, where 16.5°C selected larvae showed higher mean weights at each time interval at 16.5°C, compared to 25°C selected larvae. But why do these larvae also exhibit higher mean weights at 25°C? One might expect alleles controlling growth to be temperature-specific, in which case

larvae from each selection regime would have been heavier, but only at the temperature at which they had been selected. The higher mean weights of the 16.5°C selected larvae suggests that these larvae may be allocating more nutrients to growth than are 25°C selected larvae.

Results from starvation experiments at both growth temperatures confirm that 16.5°C selected larvae were heavier at each sampling interval than 25°C selected larvae. Growth temperature also had an effect on critical larval weights for pupariation, these being lower at the lower growth temperature for larvae from both selection regimes.

Larvae from thermally selected lines had higher critical weights when reared at the temperature at which they had been selected. Low critical weight is known to increase larval competitive ability (Bakker 1961, 1969). However larvae from the selected lines have been shown to have greater competitive ability at the temperature at which they had been selected (Chapter 5). This could not have been predicted on the basis of results from the present experiment.

Partridge and Fowler (1993) suggest that evolutionary changes in size could be due mainly to changes in critical weight with feeding rate little altered. Flies from the thermally selected lines were found to have evolved with respect to body size (Chapter 6), 16.5°C selected lines having larger thorax length than 25°C lines at both 16.5°C and 25°C. One might therefore expect 16.5°C selected lines to have the higher critical larval weights at both 16.5°C and 25°C. This is not the case. Alternatively, higher final size can be achieved through an extended larval period, but this does not happen with the

16.5°C selected lines (see Chapter 3). The greater size observed in 16.5°C lines at both growth temperatures could be due to an increased growth rate in the post-critical phase of larval development. Thermal selection may therefore be acting upon different genes than artificial selection for size at a single temperature, as lines selected for large size show no increase in larval growth rate (Robertson 1963).

The experiments reported here have shown that those thermally selected lines with greater mean weights at each sampling interval are those which also have shorter larval development (Chapter 3) and larger body size (Chapter 6) at both growth temperatures. When reared at 25°C, the 16.5°C lines have been shown to have a greater adult body size but lower critical larval weight and shorter larval development time than the 25°C lines, but still have the greater larval growth rate. Larval growth rates are therefore correlated with large adult size whereas critical larval weights seem not be. It would be interesting to find out if 16.5°C selected lines really are allocating more nutrients to growth as opposed to say somatic maintenance. This could possibly be investigated using both selected lines at both growth temperatures by limiting nutrients at several fixed levels throughout larval development. One could then examine larval weights throughout development, critical weights for pupariation and eventual adult body size in both lines to determine whether any differences existed between the selection regimes.

Figure 4.1 Male larval growth curves at 25°C
(+/- 95% confidence limits)

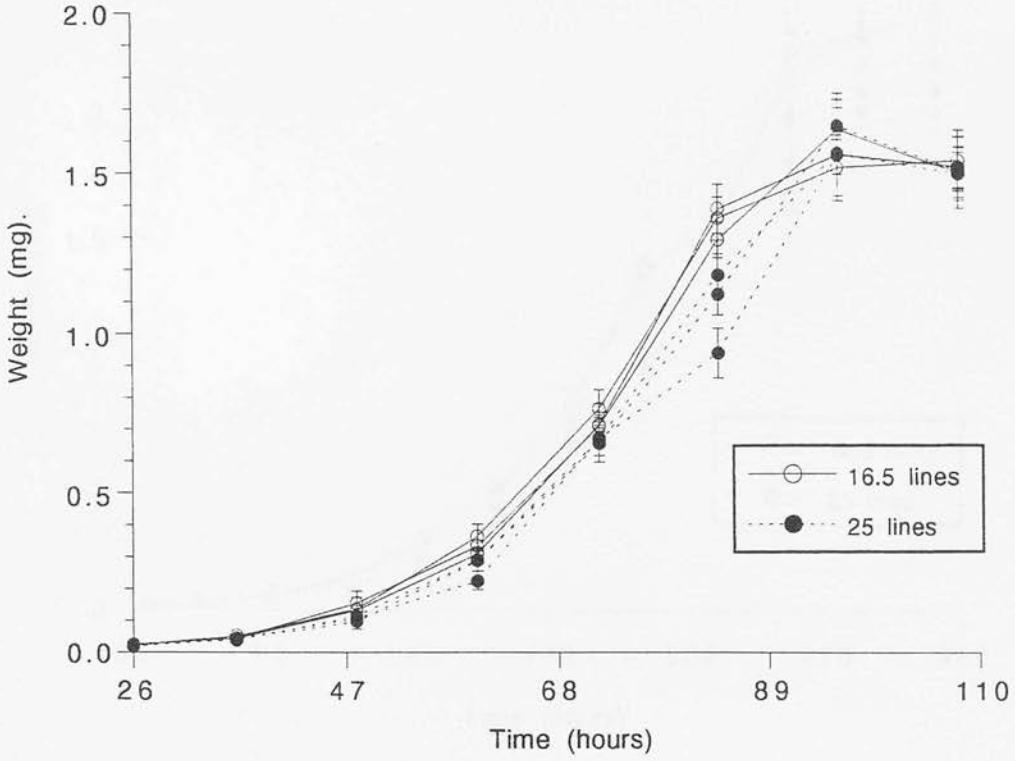


Figure 4.2 Female larval growth curves at 25°C
(+/- 95% confidence limits)

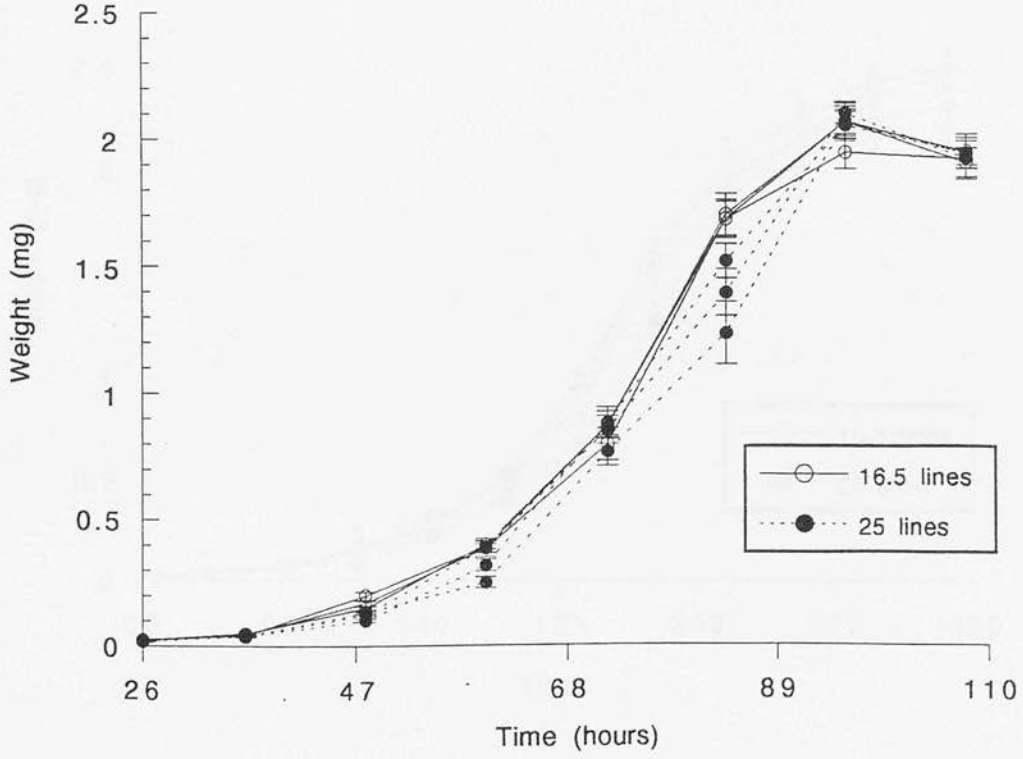


Figure 4.3 Male larval growth curves at 16.5°C
(+/- 95% confidence limits)

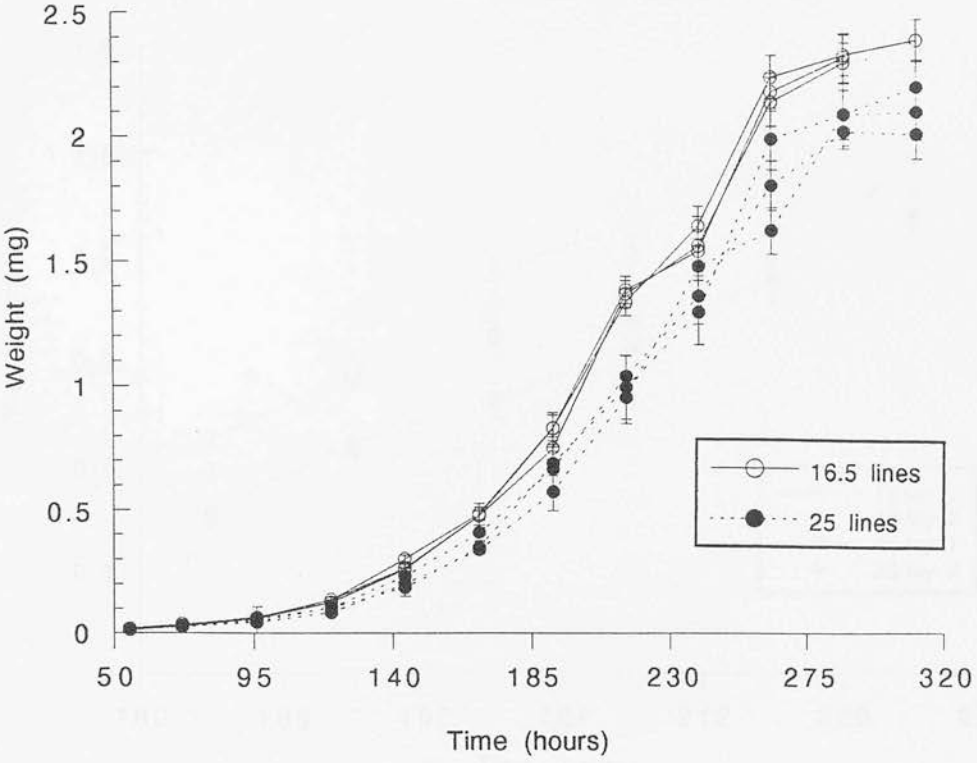


Figure 4.4 Female larval growth curves at 16.5°C
(+/- 95% confidence limits)

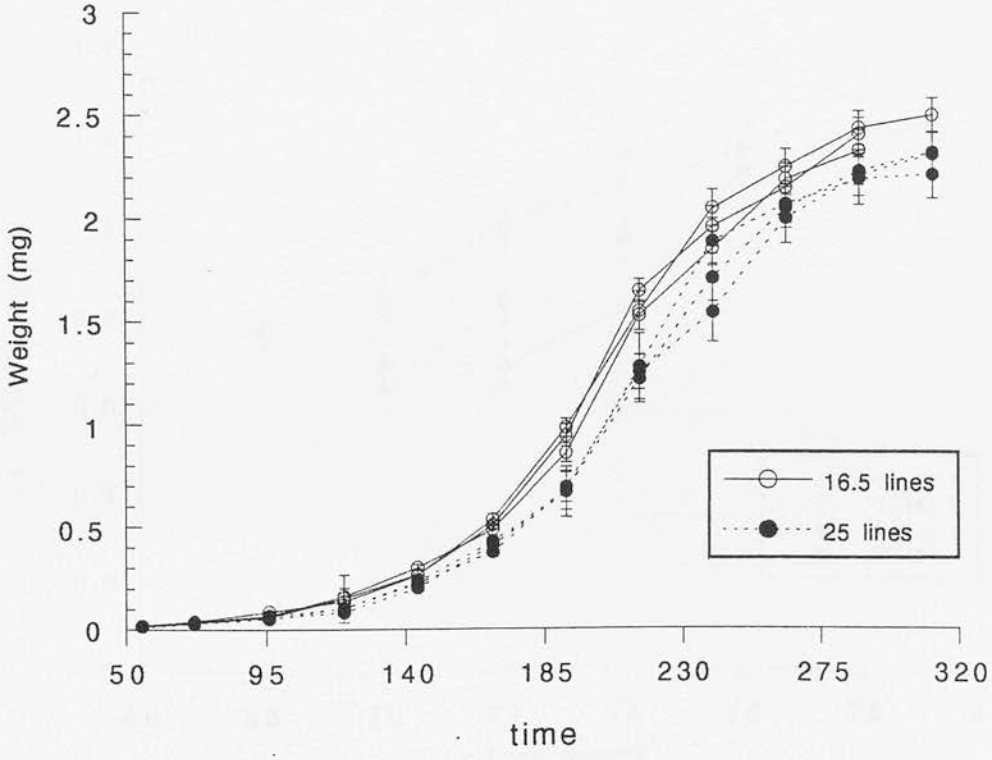


Figure 4.5 Larval weights - 16.5°C starvation experiment
(+/- 95% confidence limits)

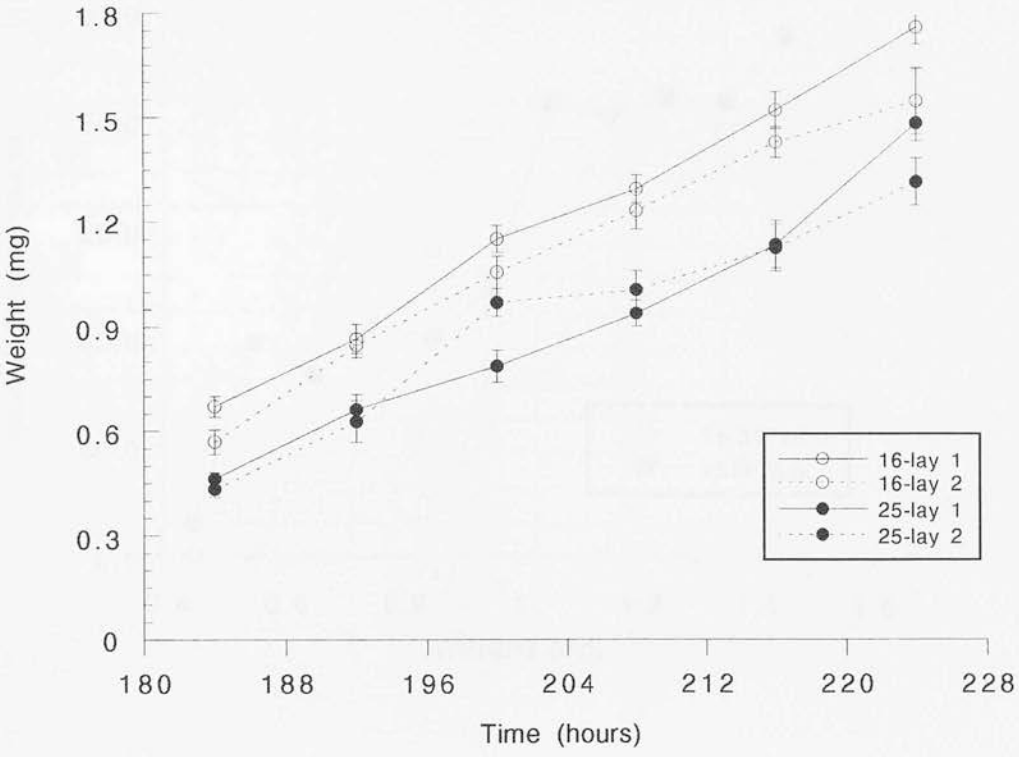


Figure 4.6 Larval weights - 25°C starvation experiment
(+/- 95 % confidence limits)

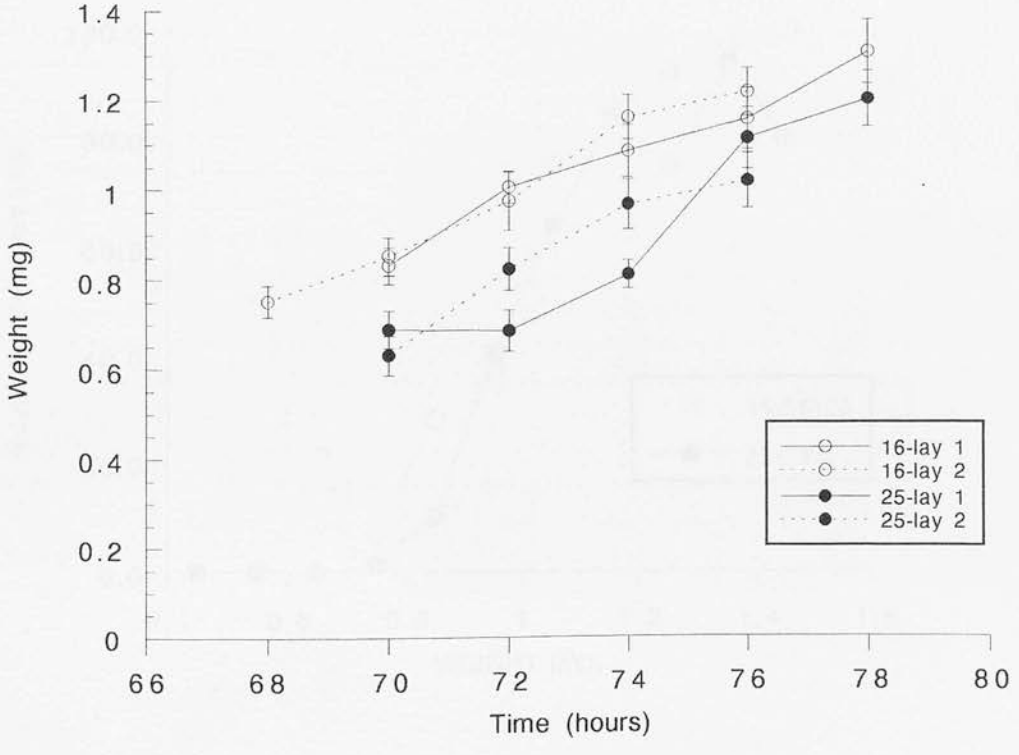


Figure 4.7 Percentage of larvae giving rise to adults - 16.5°C starvation experiment

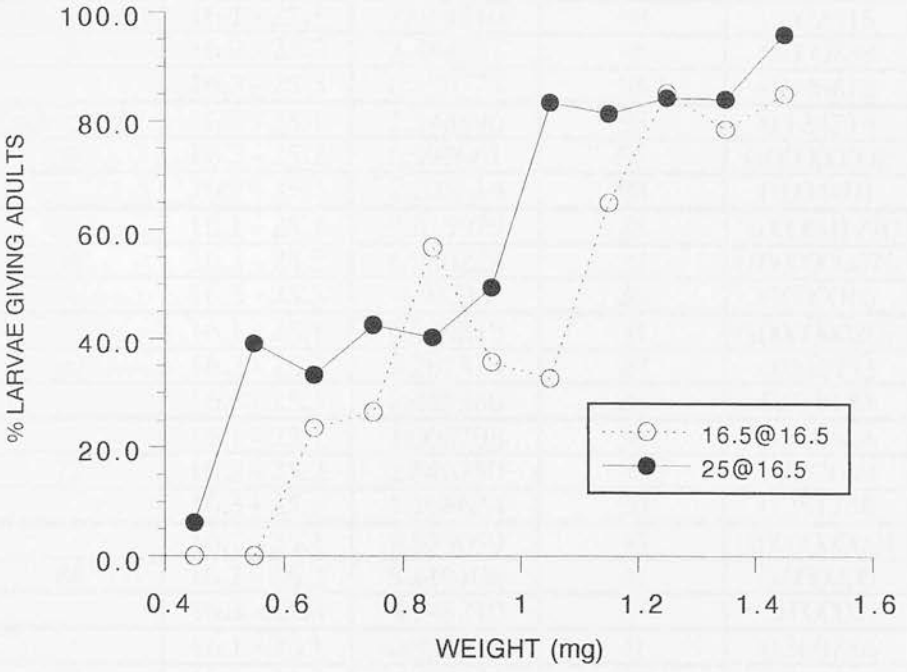


Figure 4.8 Percentage of larvae giving rise to adults - 25°C starvation experiment

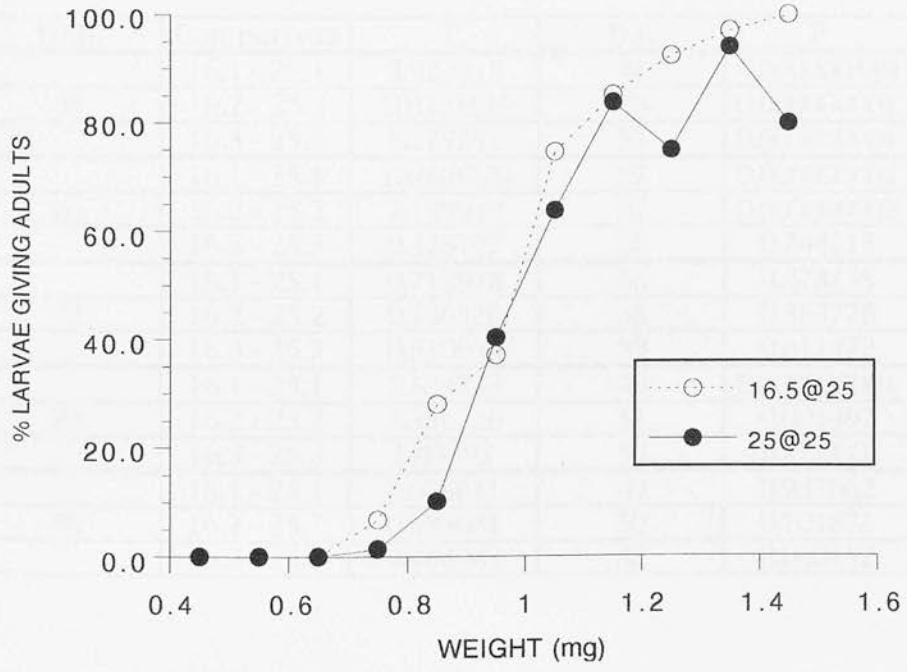


Table 4.1 25°C Growth curve analysis - males.
Multiple comparisons between selected lines

TIME	Comparison	T	D.F.	P
(unsexed)	16.1 - 25.1	3.053210	98	0.002915
26	16.2 - 25.2	3.484351	98	0.000739
	16.3 - 25.3	0.390775	98	0.696812
(unsexed)	16.1 - 25.1	1.548546	98	0.124714
36	16.2 - 25.2	6.598061	98	0.00000002
	16.3 - 25.3	2.208614	98	0.004491
	16.1 - 25.1	5.835979	35	0.000001270
48	16.2 - 25.2	6.930223	32	0.000000076
	16.3 - 25.3	4.98511	36	0.000016
	16.1 - 25.1	6.630819	31	0.000000207
60	16.2 - 25.2	3.267514	27	0.002953
	16.3 - 25.3	2.485386	33	0.018183
	16.1 - 25.1	1.008798	36	0.319804
72	16.2 - 25.2	2.846750	32	0.007649
	16.3 - 25.3	1.168624	30	0.251758
	16.1 - 25.1	8.575059	38	0.000000001
84	16.2 - 25.2	5.216106	41	0.000006
	16.3 - 25.3	4.943210	37	0.00001
	16.1 - 25.1	0.906844	41	0.369788
96	16.2 - 25.2	0.387335	42	0.700464
	16.3 - 25.3	4.605692	43	0.000036

Table 4.1(b) 25°C Growth curve analysis - females.
Multiple comparisons between selected lines

TIME	Comparison	T	D.F.	P
	16.1 - 25.1	5.922018	41	0.000000559
48	16.2 - 25.2	10.099394	46	0.000000001
	16.3 - 25.3	8.279252	53	0.000000001
	16.1 - 25.1	10.028720	57	0.000000001
60	16.2 - 25.2	7.199919	52	0.000000002
	16.3 - 25.3	0.328797	38	0.744115
	16.1 - 25.1	0.713928	58	0.478135
72	16.2 - 25.2	0.146826	58	0.883778
	16.3 - 25.3	0.510693	59	0.611472
	16.1 - 25.1	7.554733	49	0.000000001
84	16.2 - 25.2	3.356726	51	0.001497
	16.3 - 25.3	5.45392	50	0.0000001
	16.1 - 25.1	0.078611	49	0.937662
96	16.2 - 25.2	0.385081	50	0.701871
	16.3 - 25.3	4.164242	42	0.000152

Table 4.2 Combined analysis at 25°C

TIME & SEX	$\Sigma \ln P$	$-2\Sigma \ln P$	P
26 (unsexed)	-13.409338	26.818676	<0.001
36 (unsexed)	-25.214946	50.429892	<0.001
48 (males)	-41.011948	82.023896	<0.001
60 (males)	-25.222749	50.445498	<0.001
72 (males)	-7.3925143	14.785029	<0.05
84 (males)	-32.747017	65.494034	<0.001
96 (males)	-11.582829	23.165658	<0.001
48 (females)	-55.843648	111.6873	<0.001
60 (females)	-41.048944	82.097888	<0.001
72 (females)	-1.3532977	2.7065953	<0.9
84 (females)	-27.227558	54.455116	<0.001
96 (females)	-9.2100014	18.420003	<0.01

The probabilities from the indepedent tests of significance were combined using the method outlined by Sokal and Rohlf. $-2\Sigma \ln P$ is distributed as χ^2 with 2k degrees of freedom, where k= number of independent tests of

Table 4.3(a) 16.5°C Growth curve analysis - males.
Multiple comparisons between selected lines

TIME	Comparison	T	D.F.	P
(unsexed)	16.1 - 25.1	-1.031306	98	0.304936
55	16.2 - 25.2	-4.067656	98	0.000096
	16.3 - 25.3	-2.979694	98	0.003638
	16.1 - 25.1	0.233762	10	0.819886
72	16.2 - 25.2	-1.466997	15	0.163029
	16.3 - 25.3	-3.219944	9	0.010490
	16.1 - 25.1	-0.579472	23	0.567904
96	16.2 - 25.2	-3.727177	23	0.001105
	16.3 - 25.3	-5.537901	22	0.000014
	16.1 - 25.1	-2.530560	35	0.016039
120	16.2 - 25.2	-7.387346	33	0.000000017
	16.3 - 25.3	-6.435324	31	0.000000358
	16.1 - 25.1	-3.325027	26	0.002638
144	16.2 - 25.2	-12.994180	26	0.000000017
	16.3 - 25.3	-6.380415	36	0.000000216
	16.1 - 25.1	-12.452829	30	0.000000001
168	16.2 - 25.2	-2.424865	30	0.021546
	16.3 - 25.3	-14.407776	37	0.000000001
	16.1 - 25.1	-2.008801	31	0.053336
192	16.2 - 25.2	-3.055170	36	0.04219
	16.3 - 25.3	-5.922224	33	0.000001213
	16.1 - 25.1	-6.071231	38	0.000000455
216	16.2 - 25.2	-6.279069	35	0.000000332
	16.3 - 25.3	-7.448703	42	0.000000003
	16.1 - 25.1	-3.154629	41	0.003007
240	16.2 - 25.2	-4.425704	36	0.000086
	16.3 - 25.3	-7.841394	40	0.000000001
	16.1 - 25.1	-4.940435	44	0.000012
264	16.2 - 25.2	-6.940435	43	0.000000015
	16.3 - 25.3	-11.234019	47	0.000000001
	16.1 - 25.1	-3.790210	43	0.000464
288	16.2 - 25.2	-3.071324	44	0.003647
	16.3 - 25.3	-1.1933715	46	0.059315

Table 4.3(b) 16.5°C Growth curve analysis - females.
Multiple comparisons between selected lines

TIME	Comparison	T	D.F.	P
	16.1 - 25.1	-1.039563	10	0.323025
72	16.2 - 25.2	-1.809939	7	0.113225
	16.3 - 25.3	-1.238899	10	0.244740
	16.1 - 25.1	0.691743	31	0.494247
96	16.2 - 25.2	-4.654104	28	0.000071
	16.3 - 25.3	4.624431	28	0.000077
	16.1 - 25.1	-1.398991	33	0.171145
120	16.2 - 25.2	-4.024468	38	0.000263
	16.3 - 25.3	-2.38349	27	0.033642
	16.1 - 25.1	0.498235	40	0.621047
144	16.2 - 25.2	-0.033853	48	0.000007
	16.3 - 25.3	-10.545925	47	0.000000001
	16.1 - 25.1	-9.613449	41	0.000000001
168	16.2 - 25.2	-4.399473	40	0.000078
	16.3 - 25.3	-9.841743	37	0.000000001
	16.1 - 25.1	-5.692233	36	0.000002
192	16.2 - 25.2	-7.946164	52	0.000000001
	16.3 - 25.3	-3.228016	41	0.002454
	16.1 - 25.1	-7.755745	42	0.000000001
216	16.2 - 25.2	-4.697780	37	0.000036
	16.3 - 25.3	-3.383186	39	0.001643
	16.1 - 25.1	-5.045843	44	0.000008
240	16.2 - 25.2	-4.832296	54	0.000012
	16.3 - 25.3	-4.844051	45	0.000015
	16.1 - 25.1	-0.818387	47	0.417266
264	16.2 - 25.2	-6.330176	49	0.000000072
	16.3 - 25.3	-10.931750	45	0.000000001
	16.1 - 25.1	-2.989965	47	0.004430
288	16.2 - 25.2	-5.699012	50	0.00000064
	16.3 - 25.3	-2.771296	46	0.008031

Table 4.4 Combined analysis at 16.5°C

TIME & SEX	$\Sigma \ln P$	$-2\Sigma \ln P$	P
55 (unsexed)	-16.055137	32.110274	<0.001
72 (males)	-6.56975	13.1395	<0.05
96 (males)	-18.550166	37.100332	<0.01
120 (males)	-4.1327316	8.2654632	<0.5
144 (males)	-42.008987	84.017974	<0.001
168 (males)	-45.284097	90.568194	<0.001
192 (males)	-19.71913	39.43826	<0.001
216 (males)	-49.145753	98.291506	<0.001
240 (males)	-35.891241	71.782482	<0.001
264 (males)	-50.069085	100.13817	<0.001
288 (males)	-16.1143369	32.228738	<0.001
72 (females)	-4.7159627	9.4319254	<0.5
96 (females)	-19.729256	39.458512	<0.001
120 (females)	-13.400581	26.801162	<0.001
144 (females)	-33.069215	66.13843	<0.001
168 (females)	-50.905333	101.81067	<0.001
192 (females)	-39.855665	79.71133	<0.001
216 (females)	-37.366489	74.732978	<0.001
240 (females)	-34.174133	68.348266	<0.001
264 (females)	-38.043897	76.087794	<0.001
288 (females)	-22.203014	44.406028	<0.001

The probabilities from the indepedent tests of significance were combined using the method outlined by Sokal and Rohlf. $-2\Sigma \ln P$ is distributed as χ^2 with 2k degrees of freedom, where k= number of independent tests of significance.

Table 4.5(a) Number of larvae not producing/producing adults within each age and weight category.
25°C experiment - 16.5°C lines.

	WEIGHT CATEGORY							
TIME	0.7 - 0.8	0.8 - 0.9	0.9 - 1.0	1.0 - 1.1	1.1 - 1.2	1.2 - 1.3	1.3 - 1.4	1.4 - 1.5
70	28 / 3	18 / 2	14 / 9	3 / 4	1 / 2	0 / 1	-	-
72	7 / 0	11 / 8	11 / 6	10 / 17	5 / 7	3 / 6	0 / 3	0 / 1
74	3 / 0	3 / 4	6 / 8	3 / 18	0 / 17	0 / 18	1 / 11	0 / 5
76	2 / 0	4 / 0	9 / 3	2 / 12	2 / 19	1 / 18	0 / 10	0 / 7
78	1 / 0	-	4 / 0	0 / 2	1 / 7	0 / 6	0 / 9	0 / 8

Table 4.5(b) Percentage of larvae giving rise to adults within each age and weight category.
25°C experiment - 16.5°C lines.

	WEIGHT CATEGORY							
TIME	0.7 - 0.8	0.8 - 0.9	0.9 - 1.0	1.0 - 1.1	1.1 - 1.2	1.2 - 1.3	1.3 - 1.4	1.4 - 1.5
70	9.7%	10%	39.1%	57.1%	67%	100%	-	-
72	0%	42.1%	35.3%	63%	58.3%	67%	100%	100%
74	0%	57.1%	57.1%	85.7%	100%	100%	91.7%	100%
76	0%	0%	25%	85.7%	90.5%	94.7%	100%	100%
78	0%	-	0%	100%	87.5%	100%	100%	100%

Table 4.6(a) Number of larvae not producing/producing adults within each age and weight category.
25°C experiment - 25°C lines

	WEIGHT CATEGORY							
TIME	0.7 - 0.8	0.8 - 0.9	0.9 - 1.0	1.0 - 1.1	1.1 - 1.2	1.2 - 1.3	1.3 - 1.4	1.4 - 1.5
70	18 / 0	14 / 0	1 / 1	1 / 0	-	0 / 1	-	-
72	23 / 0	18 / 1	6 / 2	4 / 5	0 / 2	-	-	-
74	29 / 0	15 / 2	15 / 6	7 / 4	1 / 5	1 / 2	0 / 2	-
76	2 / 1	3 / 3	8 / 12	3 / 11	3 / 14	2 / 11	1 / 9	0 / 2
78	1 / 0	3 / 0	1 / 0	2 / 10	2 / 10	3 / 4	0 / 5	1 / 2

Table 4.6(b) Percentage of larvae giving rise to adults within each age and weight category.
25°C experiment - 25°C lines.

	WEIGHT CATEGORY							
TIME	0.7 - 0.8	0.8 - 0.9	0.9 - 1.0	1.0 - 1.1	1.1 - 1.2	1.2 - 1.3	1.3 - 1.4	1.4 - 1.5
70	0%	0%	50%	0%	-	100%	-	-
72	0%	5.3%	25%	55.6%	100%	-	-	-
74	0%	11.8%	28.6%	36.4%	83.3%	67%	100%	-
76	33%	50%	60%	78.6%	82.3%	84.6%	90%	100%
78	0%	0%	0%	91.7%	91.7%	57.1%	100%	66.7%

Table 4.7(a) Number of larvae not producing/producing adults within each age and weight category. 16.5°C experiment - 16.5°C lines.

	WEIGHT CATEGORY										
TIME	0.4 - 0.5	0.5 - 0.6	0.6 - 0.7	0.7 - 0.8	0.8 - 0.9	0.9 - 1.0	1.0 - 1.1	1.1 - 1.2	1.2 - 1.3	1.3 - 1.4	1.4 - 1.5
184	12 / 0	28 / 0	21 / 8	8 / 10	0 / 8	-	-	-	-	-	-
192	-	4 / 0	5 / 0	26 / 3	11 / 17	4 / 15	1 / 8	0 / 4	0 / 1	-	-
200	-	-	0 / 0	2 / 0	7 / 0	16 / 0	22 / 10	2 / 16	0 / 19	0 / 3	-
208	-	-	-	-	1 / 0	4 / 0	13 / 1	9 / 6	0 / 24	2 / 21	0 / 6
216	-	-	-	-	-	-	-	3 / 1	8 / 6	8 / 11	3 / 20
224	-	-	-	-	-	3 / 0	-	1 / 1	1 / 3	1 / 6	3 / 9

Table 4.7(b) Percentage of larvae giving rise to adults within each age and weight category. 16.5°C experiment - 16.5°C lines.

	WEIGHT CATEGORY										
TIME	0.4 - 0.5	0.5 - 0.6	0.6 - 0.7	0.7 - 0.8	0.8 - 0.9	0.9 - 1.0	1.0 - 1.1	1.1 - 1.2	1.2 - 1.3	1.3 - 1.4	1.4 - 1.5
184	0%	0%	27.6%	55.6%	100%	-	-	-	-	-	-
192	-	0%	0%	10.3%	60.7%	78.9%	88.9%	100%	100%	-	-
200	-	-	-	0%	0%	0%	31.3%	88.9%	100%	100%	-
208	-	-	-	-	0%	0%	17.1%	40%	100%	91.3%	100%
216	-	-	-	-	-	-	-	25%	42.9%	57.9%	87%
224	-	-	-	0%	-	0%	-	50%	75%	85.7%	75%

Table 4.8(a) Number of larvae not producing/producing adults within each age and weight category. 16.5°C experiment - 25°C lines.

	WEIGHT CATEGORY										
TIME	0.4 - 0.5	0.5 - 0.6	0.6 - 0.7	0.7 - 0.8	0.8 - 0.9	0.9 - 1.0	1.0 - 1.1	1.1 - 1.2	1.2 - 1.3	1.3 - 1.4	1.4 - 1.5
184	47 / 4	5 / 17	1 / 0	-	-	-	-	-	-	-	-
192	14 / 0	16 / 0	13 / 13	4 / 13	2 / 7	0 / 6	0 / 2	-	-	0 / 1	-
200	-	6 / 1	10 / 0	7 / 3	14 / 10	9 / 13	1 / 18	0 / 5	0 / 2	-	-
208	-	1 / 0	-	11 / 1	13 / 8	12 / 16	4 / 17	0 / 9	0 / 6	0 / 1	-
216	-	-	2 / 0	1 / 0	9 / 2	17 / 5	1 / 11	1 / 14	0 / 10	1 / 11	0 / 8
224	-	-	-	-	2 / 0	3 / 0	4 / 3	6 / 3	4 / 4	4 / 14	1 / 19

Table 4.8(b) Percentage of larvae giving rise to adults within each age and weight category. 16.5°C experiment - 25°C lines - percentages.

	WEIGHT CATEGORY										
TIME	0.4 - 0.5	0.5 - 0.6	0.6 - 0.7	0.7 - 0.8	0.8 - 0.9	0.9 - 1.0	1.0 - 1.1	1.1 - 1.2	1.2 - 1.3	1.3 - 1.4	1.4 - 1.5
184	7.8%	22.7%	0%	-	-	-	-	-	-	-	-
192	0%	0%	50%	76.5%	77.8%	100%	100%	-	-	100%	-
200	-	14.3%	0%	30%	41.7%	59%	94.7%	100%	100%	-	-
208	-	0%	-	8.3%	38.1%	57.1%	80.9%	100%	100%	100%	-
216	-	-	0%	0%	18.2%	22.7%	91.7%	93.3%	100%	91.7%	100%
224	-	-	-	-	0%	0%	42.9%	33.3%	50%	77.8%	95%

Chapter 5 LARVAL COMPETITIVE ABILITY

5.1 Introduction

With respect to food supply in the wild, Drosophila larvae are "scramble" type competitors, exploiting often transient food sources (Godoy-Herrera et al. 1984). It is known that larvae of several Drosophila species can discriminate between different strains of yeast, and between different humidity levels (Benz 1956; Lindsay 1958; Cooper 1960) and between levels of ethanol in food medium (Parsons 1977; 1979, Cavener 1979; Gelfand and McDonald 1980). Levels of sucrose and the presence of other larvae are also known to elicit attraction/avoidance behaviours in Drosophila larvae (Pruzan and Bush 1977; Miyakawa et al. 1980). All the above behaviours will contribute, to a greater or lesser extent, to the overall competitive ability of the larvae under different environmental conditions.

D. melanogaster larvae show a variety of behaviours associated with competition, feeding and the selection of suitable sites for pupation. Green et al. (1983) measured the rates of locomotor activity in D. melanogaster and D. simulans larvae, describing a number of discrete components of behaviour. They observed an increase in locomotor activity on non-nutritive substrates, accompanied by increased 'exploratory movements', which together constituted foraging. When in contact with food the predominant activity changed to feeding, involving the rhythmic extension and contraction of the mouth hooks. Larvae also showed an increased tendency to dig into the substrate in the presence of food (Godoy-Herrera 1977; 1978). Locomotor

activity increased and feeding behaviour decreased when larvae were exposed to elevated concentrations of ethanol, possibly an adaptive response to unfavourable environments (Green et al. 1983). By moving rapidly over non-nutritive or unfavourable substrates, larvae could maximise the time spent in any available favourable environments (Green et al. 1983; Godoy-Herrera et al. 1984).

There is a generally recognised division of larval behavioural patterns in D. melanogaster, associated with the adaptations of the larva to the trophic and metamorphic phases of its life history (e.g. Burnet et al 1977; Sokolowski 1980; Green et al. 1983; Godoy-Herrera 1984). Grossfield (1978) describes a switch in 'programmed preference', when, in contrast to the earlier instars (whose preference is for moist substrates), late third instar larvae show a preference for drier substrates, just before pupariation. The temporal relationships between this switch to preference for a drier substrate, the changes in geotactic and phototactic responses, and the cessation of feeding activity, remain unclear.

As larvae develop, the demand for food gets greater, increasing potential competition between individuals. When the food resource has been depleted at one location, greater locomotor activity may extend the foraging range. So, it would seem advantageous for locomotor activity to increase with age, and this does indeed happen. Although this may be largely a function of gain in body size with larval age, the results of Godoy-Herrera et al. (1984) suggest that there may be an age related change in locomotor activity.

Several species of Drosophila are known to coexist in the same feeding site (Atkinson and Shorrocks 1977; Atkinson 1979). However, some form of resource partitioning usually takes place (Atkinson and Shorrocks 1977), by differential larval burrowing behaviour (Barker 1971; Arthur and Middlecote 1987) or through a succession of species arriving at the food source (Lachaise et al. 1982; Nunney 1990). Many field studies of Drosophila have inferred the existence of larval competition, even during plentiful food supply (McKenzie and Parsons 1972; Budnick and Brncic 1974).

Atkinson (1979) investigated the occurence of larval competition in several domestic species of Drosophila at Leeds fruit and vegetable market, and regular seasonal changes in body size were demonstrated. When the heritability of body size in the field was examined it was found to be negligible. therefore the seasonal changes were due to environmental effects, such as temperature and larval crowding.

Atkinson & Shorrocks (1977) found a significant correlation between the numbers of D. melanogaster emerging from field breeding sites returned to the laboratory in a particular week and the mean weekly temperature. They concluded that larvae developing at higher temperatures were more likely to be crowded. The seasonal changes in body size shown by Atkinson (1979) may therefore be due to differing degrees of larval competition as well as the physiological effects of temperature. Simple regression analysis showed a highly significant negative correlation between wing length and temperature and a very much smaller, but significant negative correlation between wing length and density. In order to determine whether larval density

was as unimportant as the simple regression suggested, or whether its effects were masked by the effect of temperature, Atkinson (1979) carried out a multiple regression analysis to separate the effects of temperature from the effects of density. The number of D. melanogaster in each breeding site was found to account for a significant amount of the variation in body size. Atkinson (1979) concluded that there was some limiting resource preventing the larvae growing to a size determined by temperature; the larvae must be competing.

Before Atkinson (1979), most studies on body size in Drosophila had not attempted to disentangle the effects of temperature from those of food shortage, but concluded simply that temperature was the most important environmental factor influencing adult body size (e.g. Stalker and Carson 1947; Sokoloff 1957; Tantaway 1964; McFarquhar & Robertson 1963; Sokoloff 1966). The results presented by Atkinson (1979) however demonstrated a correlation between temperature and larval crowding which concealed the effects of larval competition on body size. Atkinson's conclusion was that larval competition in Drosophila may be much more common than previous studies suggested. More recent studies have confirmed that both intraspecific and interspecific larval competition are common in field conditions (e.g. Atkinson 1985; Grimaldi & Jaenike 1984; Prout & Barker 1989; Nunney 1990).

Adult body size has been shown to have a significant effect on fitness in Drosophila, with longevity, female fertility and male mating success all increased (Tantaway & Vetukhiv 1960; Tantaway & Rakha 1964: Partridge & Farquhar 1981, 1983; Partridge et al. 1987a, 1987b; Santos et al. 1988; 1992b).

However, artificial selection for increased thorax length in Drosophila has been shown to cause a decrease in preadult viability at medium and high larval densities (Santos et al. 1992a; Partridge & Fowler 1993). The eventual body size of adult Drosophila may be an evolutionary compromise between the beneficial effects of large adult size and the disadvantage of the associated reduction in larval survival. In stocks artificially selected for body size, pre adult survival may decrease with large size because of an associated increase in larval development times (Reeve 1954; Sang 1956; Robertson 1957, 1960, 1963; Partridge & Fowler 1993). An extended developmental period could increase larval mortality simply through higher cumulative mortality (any mortality at older ages penalises prolonged development). Also, if breeding site conditions deteriorate with time (dehydration, acumulation of toxic residues, removal of nutrients, increased bacterial and fungal growth, increased predation), then mortality will increase with the age of the breeding site (e.g. Dawood & Strickberger 1969; Budnik & Brncic 1975; Botella et al. 1985). Santos et al. (1992a) and Partridge & Fowler (1993) tested for a genetic correlation between adult size and preadult survival by measuring preadult survival in lines artificially selected for thorax length. They have shown that preadult survival of large selected lines decreased disproportionately to that of control and small lines as larval density was increased. The reduced viability of the large lines may have been a consequence of high critical weight (see Chapter 4), since the two major factors known to increase larval competitive ability are low critical weight and high feeding rate (Bakker 1961, 1969). Furthermore, selection

for large size extended the larval period, but selection for small size did not reduce the larval period. An extended larval period can affect fitness in Drosophila not only through increased preadult mortality, but also by delaying first breeding. This is particularly important during periods of population expansion, when rapid larval development and consequently early breeding are advantageous (e.g. Cole 1954: Lewontin 1965).

In addition, Partridge & Fowler (1993) measured the preadult survival of the large and control lines at three different temperatures (18°C, 25°C & 28°C) to test for any interaction between size and temperature in determining preadult survival. Large lines had lower preadult survival than controls across all three temperatures. There was also a highly significant effect of temperature, with all lines having significantly lower preadult survival at 18°C than at the other two temperatures. There was no evidence for a significant interaction between size and temperature. Thermal selection, which produces an evolutionary increase in body size in response to reduced temperature, may operate through a fitness component other than preadult survival or involve different genes than those involved in size selection at a single temperature (Partridge & Fowler 1993).

The experiments reported in this chapter examine the competitive ability of larvae from both thermal selection regimes, reared at either 16.5°C or 25°C and competed against standard larvae from the sparkling poliert stocks (see Chapter 2).

5.2 Materials and Methods

The aim of these experiments was to examine the competitive ability of larvae from the two thermal selection regimes when reared at either 16.5°C or 25°C, in competition with larvae from the sparkling poliert stocks (See Chapter 2, Fig 2.2). The sparkling poliert mutation has no known effect on larval competitive ability (Lindsley and Grell 1968). Adult flies were obtained from all three cages of each selection regime and from the 16.5°C and 25°C sparkling poliert cages, as described in Chapter 3.2. The parents of the larvae used in the experiments had therefore been raised at the appropriate experimental temperatures.

Parental flies were placed into grape-juice containers. Each day for 10 days a series of 'prelays' and a 'lay' were made. Early first instar larvae were picked from the appropriate 'lay' lids every day and placed into vials containing 3.5ml of agar medium topped with 3.5ml of S-Y medium (see Chapter 2 for further details of food recipes). This combination of agar and S-Y medium reduced the amount of food but not the overall volume of the medium. The aim was to increase larval competition, while maintaining humidity in the vial and without the burden of having to pick greater numbers of first instar larvae. Varying the amount of yeast available to each developing larva is essentially equivalent to varying larval density at a constant amount of added yeast per culture vial (Economos et al. 1982; Economos and Lints 1984a; 1984b). Three classes of vials were set up, each containing 1/3 wild type larvae and 2/3 sparkling poliert larvae, at different densities as shown in Table 5.1. The

16.5°C sparkling poliert stock was used for experiments at 16.5°C and the 25°C sparkling poliert stock was used for experiments at 25°C. One vial from each thermal selection regime replicate population was set up each day, giving 6 low, 6 medium and 6 high density vials at each developmental temperature on each day for 10 days.

Table 5.1 Numbers of larvae used in experiments each day, at each density.

Vial	Wild type/ Sparkling poliert	TOTAL
Low density	33/66	99
Medium density	66/132	198
High density	132/264	396

5.3 Results

The competitive ability of the larvae from the selection regimes was calculated as: number of wild type adults emerging / (number of wild type adults + number sparkling poliert adults emerging) x 100. Figures 5.1(a)-(c) show the mean competitive abilities and 95% confidence limits for larvae reared at low, medium and high density at 16.5°C. The corresponding data for competition at 25°C are shown in Figures 5.2(a)-(c). The data for each density at 16.5°C and 25°C are given in Appendix B Tables B1(a)-(c). and B2(a)-(c) respectively.

An analysis of variance (with cage lines nested within each of the two selection regimes) was used at each developmental temperature to examine the effects of selection temperature, density, replicate lines within selection regime and their interaction on larval competitive abilities. The results are shown in Table 5.2 for 16.5°C and Table 5.3 for 25°C.

At 16.5°C there was a significant main effect of density. Comparison of means revealed highly significant differences between low & medium, medium & high, and low & high densities at 16.5°C ($P < 0.0001$ in all three comparisons), with larval competitive ability decreasing with increasing density. There was a significant main effect of selection temperature with competitive ability of larvae from the 16.5°C selection regime being greater across all three densities than that of larvae from the 25°C selection regime. The interaction term for density x selection regime was also significant. This shows that the relative competitive abilities of larvae from the two selection regimes does change with density.

At 25°C, the effect of density on larval competitive ability was again highly significant, as can be seen from the main effect of density in Table 5.3 and by comparison of means which reveals highly significant differences between low & medium, medium & high, and low & high densities at 25°C ($P < 0.0001$ in all three comparisons) Again, inspection of Figure 5.2 showed that competitive ability decreased with increasing larval density. Inspection of Figure 5.2. suggests that at each density, larvae from the 25°C selection regime showed greater competitive ability than the 16.5°C selected larvae. There was again a significant interaction between density and selection, with the

difference between 16.5°C and 25°C replicates greatest at the low density and least at the high density.

5.4 Discussion

Although there have been numerous studies of larval competitive ability, the experiment reported in this Chapter is the first examination of the competitive ability of larvae which have undergone long term thermal selection in the laboratory.

Larvae clearly showed divergence in competitive ability, with respect to thermal selection regime. At 16.5°C, larvae from the 25°C selection regime had the poorer competitive ability at all three densities, compared to 16.5°C selected larvae, when both were competed against a mutant sparkling poliert stock The situation was reversed at 25°C developmental temperature, with 25°C selected lines having greater larval competitive ability than 16.5°C selected lines when both were competed against the mutant stock.

At both 16.5°C and 25°C, the differences in larval competitive ability between the selection regimes was greatest at the lowest densities, and decreased with increasing larval density. This is a puzzling result, as one would expect that, as larval density increased, those larvae better adapted to that thermal environment would outcompete their competitors from the other thermal selection regime even more, as the challenge increased. It could be that the higher larval densities chosen in the experiment were lethal for larvae from both selection regimes. Alternatively, some other uncontrolled factor associated with the high larval densities may have adversely affected the

outcome of the experiment. For example, very high levels of uric acid and other toxic metabolic by-products, or simply lack of food, could be responsible. If any non specific factor was affecting the experiment, reducing the differences in competitive ability between the selected lines observed at lower densities, then these factors must act differentially to affect the more successful lines at each growth temperature.

Several previous papers have suggested that growth to a large adult body size could lower larval viability and have shown that in stocks artificially selected for body size, there is a genetic correlation between adult size and duration of the pre-adult period (e.g. Reeve 1954; Sang 1956; Robertson 1957, 1960, 1963). A larger adult body size could be achieved through an extended developmental period. This may result in higher pre-adult death rates, possibly because of prolonged and increased contact with deteriorating environmental conditions, perhaps due to addition of toxic wastes or removal of food (Dawood and Strickberger 1969; Budnik and Brncic 1974, 1975; Nunney 1983; Botella et al. 1985; Moya and Botella 1985; Moya et al. 1986; Castro et al. 1987). Alternatively, pre-adult death rates may increase with prolonged larval development, simply because of increased cumulative larval mortality.

The 16.5°C selected lines showed shorter development times and greater larval viability than 25°C selected lines when reared at 16.5°C. When reared at 25°C, however, the 16.5°C selected lines showed longer development times and lower larval viability than 25°C selected lines (Chapter 3). Similarly, the competitive ability of 16.5°C selected larvae was greater than 25°C selected larvae, when reared at 16.5°C. The

reverse was true at 25°C. This would seem to support the proposition that the greater pre-adult mortality observed in 16.5°C selected lines reared at 25°C is not just a result of longer development times, but also of lower larval competitive ability. The same can be said for 25°C selected lines reared at 16.5°C.

In addition to having an influence on development times and pre-adult viabilities, having a greater competitive ability should increase a larva's chance of finding sufficient food to complete development and attain target size (see Chapter 4). This will have a direct effect on eventual adult body size, the subject of Chapter 6.

Several studies have shown that large adult flies may be favoured by natural selection. For instance, large females are known to be more fecund than smaller females (Robertson 1957) and large males from a wild type population of D. melanogaster have been shown to outcompete small males for copulations and inseminate more females (Partridge and Farquar 1983).

However, artificial selection experiments have demonstrated that genetically larger flies are more likely to suffer increased mortality as eggs, pupae or larvae. This may be because genetically large flies take longer to develop (Robertson 1960), and are exposed to toxic metabolic residues which decrease or arrest larval growth (e.g. Botella et al. 1985; Moya et al. 1986). Partridge & Fowler (1993), have shown that D. melanogaster selected for large size produced fewer adults when in competition with an outbred stock with the sparkling poliert mutation, compared to flies from unselected control lines.

The experiments reported here and in Chapters 3 and 6 have shown that those flies with greater larval competitive

ability and shorter development time can nevertheless have greater body size (16.5°C lines reared at 16.5°C). However, when reared at 25°C, the 16.5°C lines still have a greater body size, but now have lower competitive abilities and longer development times compared to 25°C selected lines. This difference between the results reported here and Partridge & Fowler (1993) suggests that thermal selection involves different genes from those subject to selection for size at a single temperature. Larval mortality and competitive ability are therefore correlated with development time but not necessarily with adult size. Thermal selection may be acting on a range of inter-related fitness characters, one of which has been shown to be larval competitive ability.

Figure 5.1(a) Mean competitive ability of larvae at 16.5°C
- low density

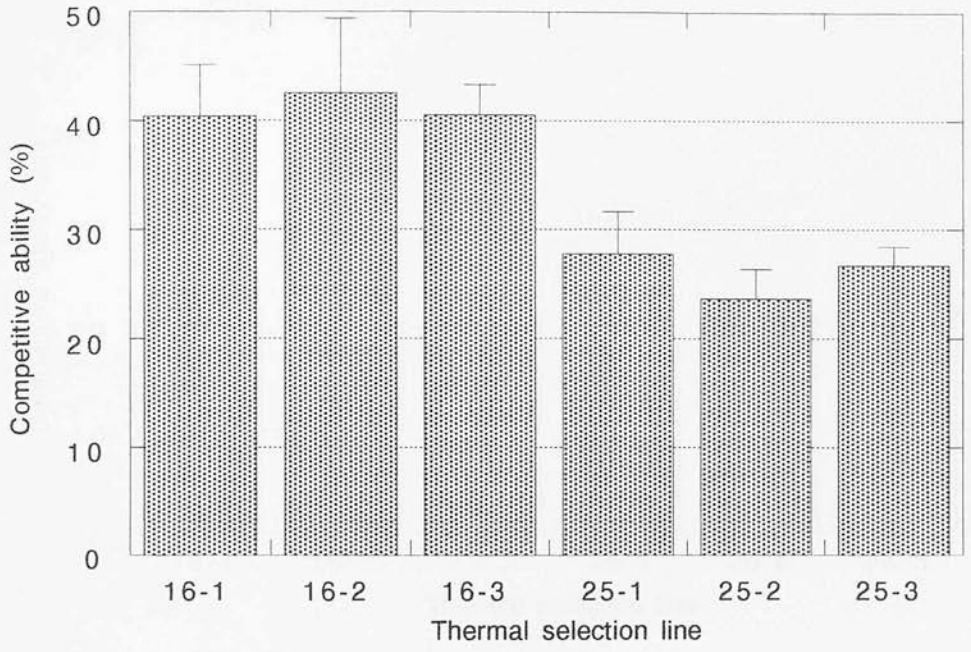


Figure 5.1(b) Competitive ability of larvae at 16.5°C
-Medium density

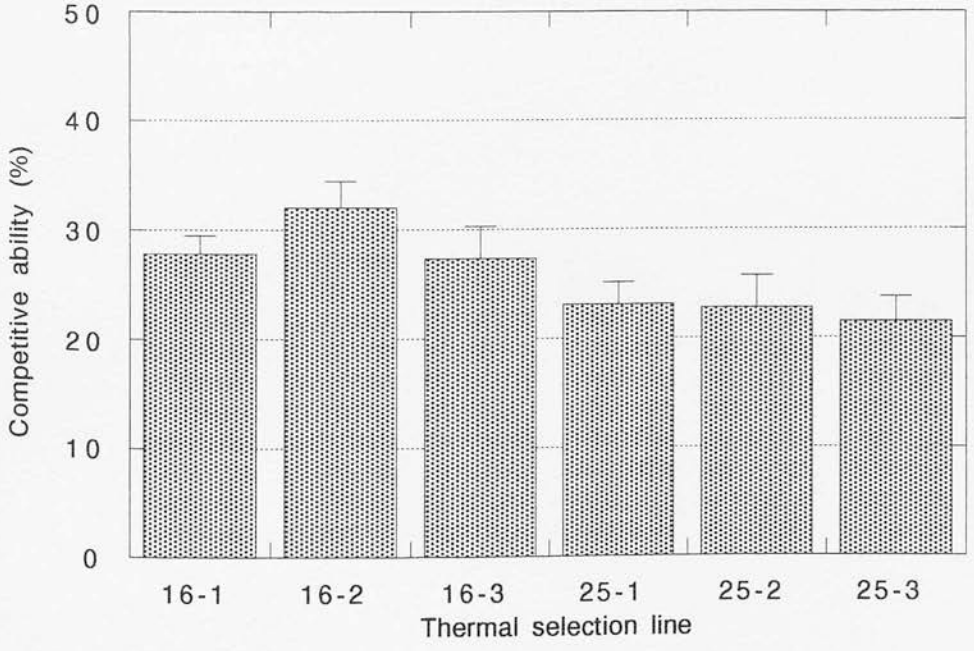


Figure 5.1(c) Competitive ability of larvae at 16.5^{0}C
- High density

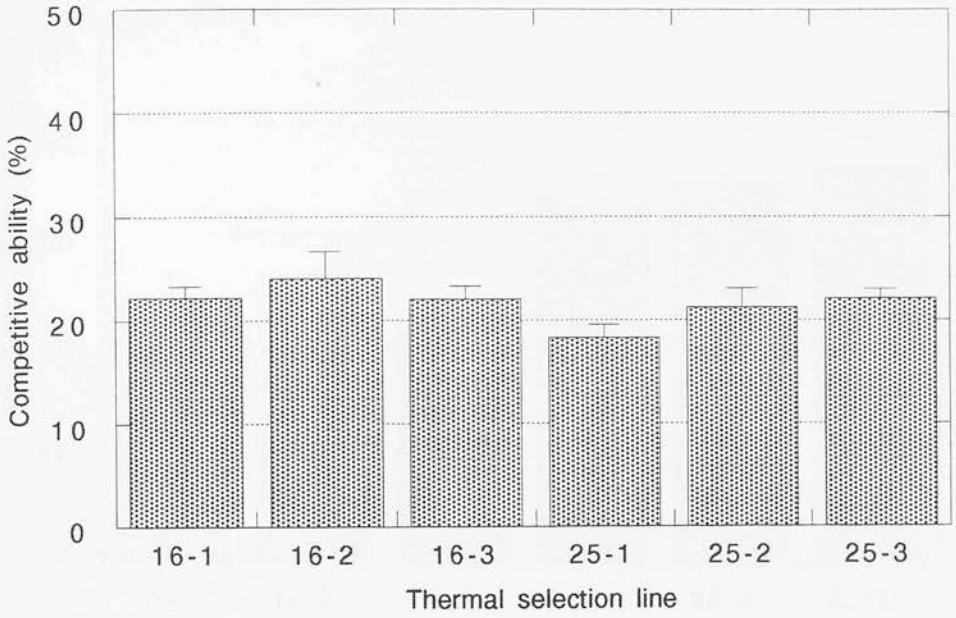


Figure 5.2(a) Competitive ability of larvae at 25°C
- Low density

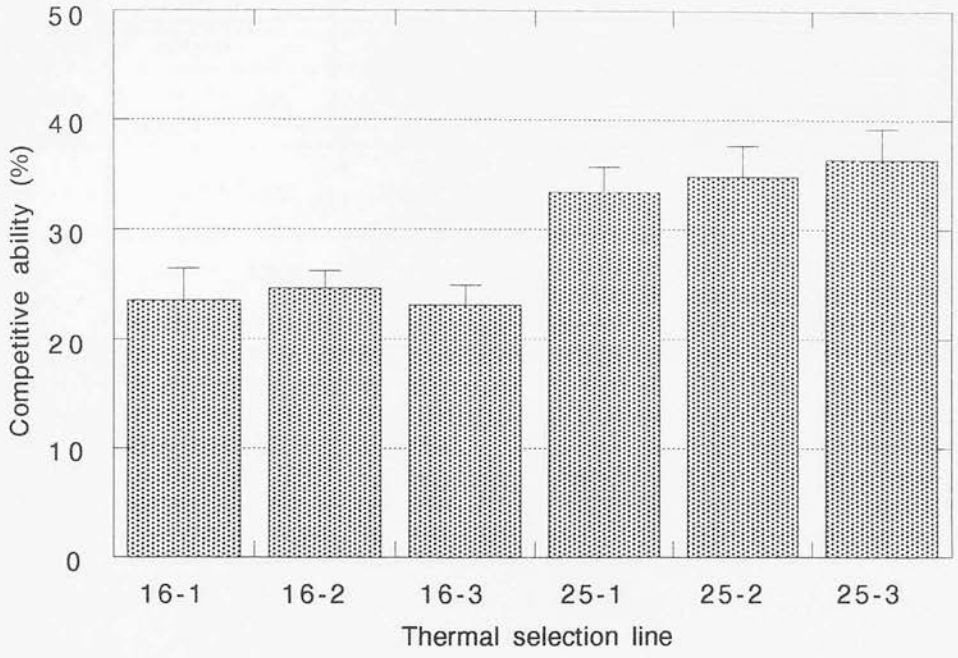


Figure 5.2(b) Competitive ability of larvae at 25°C
- Medium density

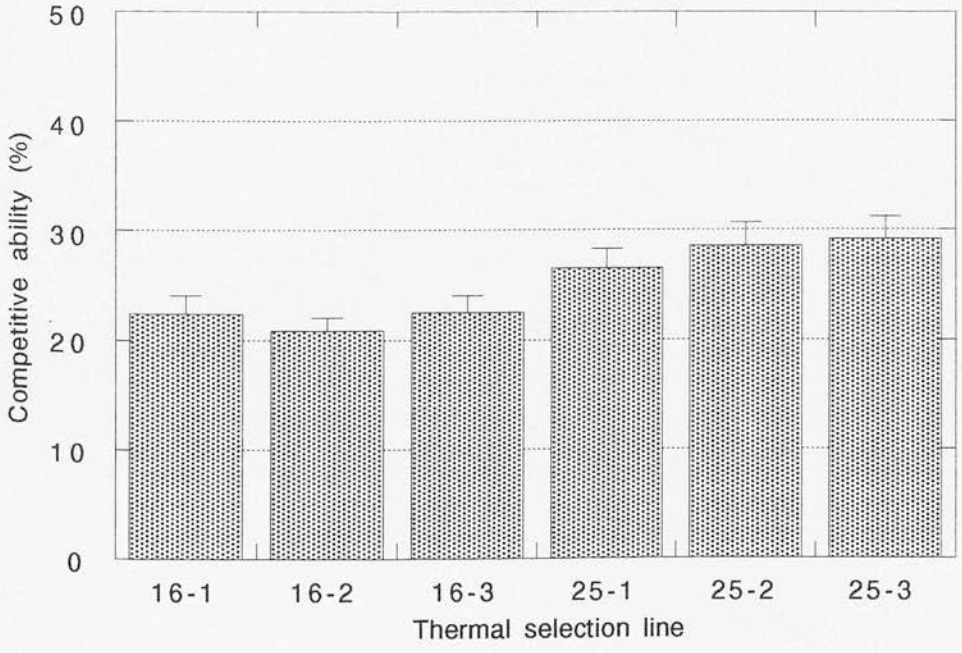


Figure 5.2(c) Competitive ability of larvae at 25°C
- High density

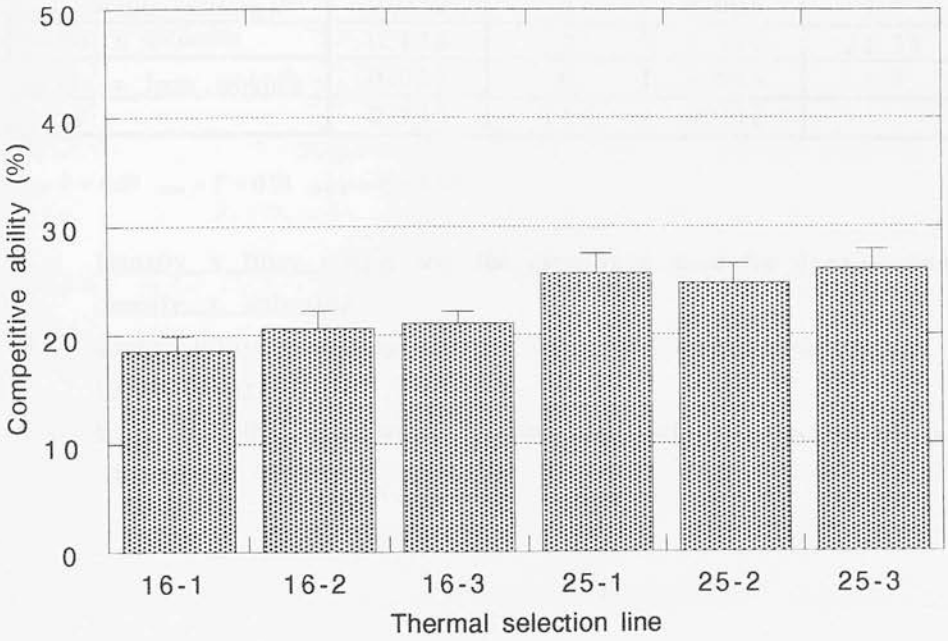


Table 5.2 NESTED ANALYSIS OF VARIANCE
- at 16.5°C growth temperature

source	ss	df	ms	F	P
density	0.541	2	0.270	90	***
selection temperature	0.324	1	0.324	64.8	**
lines within selection[B]	0.020	4	0.005	2.5	*
density x selection	0.134	2	0.067	22.33	***
density x lines within[A]	0.022	8	0.003	1.5	N.S.
error[C]	0.335	162	0.002		

* = P < 0.05 ** = P < 0.01 *** = P < 0.001

A - Density x lines within was the error term used for density and density x selection

B - Lines within selection was the error term used for selection temperature

C - Error was the error term used for lines within selection and density x selection

Table 5.3 NESTED ANALYSIS OF VARIANCE
- at 25°C growth temperature

source	ss	df	ms	F	P
density	0.157	2	0.079	39.5	***
selection temperature	0.328	1	0.328	164	***
lines within selection[B]	0.009	4	0.002	2	N.S.
density x selection	0.027	2	0.014	7	*
density x lines within[A]	0.013	8	0.002	2	*
error[C]	0.160	162	0.001		

* = P < 0.05 ** = P < 0.01 *** = P < 0.001

Error terms as in Table 5.2

Chapter 6 THE EFFECT OF THERMAL SELECTION ON ADULT BODY SIZE AND CELL SIZE

6.1 Introduction

The experiments reported in this chapter examined the developmental and evolutionary responses of body size to thermal selection in the replicated populations of *D. melanogaster* already described (Chapter 2).

As detailed in section 1.4, clinal variation in body size in *Drosophila* species has been frequently reported, and as in many other ectotherms, larger individuals are found at higher latitudes and altitudes. Geographical clines in other *Drosophila* life history characters are also well documented (section 1.5 and 1.6). Rearing different geographic populations of *Drosophila* under controlled laboratory conditions has shown that part of this clinal variation is genetic. When reared at a common temperature flies from higher latitudes are larger than those from lower latitudes. These studies, discussed in section 1.7, suggest temperature as an important selective agent on *Drosophila* body size.

There is also a purely developmental component to temperature-related body size differences in *Drosophila*. Adult flies from a given genetic stock are larger when reared at lower developmental temperatures (see section 1.8).

The similarity between these genetic and environmental components of clinal variation for body size in *Drosophila* suggests that the developmental response is an example of adaptive phenotypic plasticity (Stearns 1989; Kirkpatrick and Lofsvold 1992).

The demonstrated genetic basis for the size clines in Drosophila, and their repeatability between different species and different continents, imply that natural selection is the cause. This has been taken by many authors as a clear indication of the importance of temperature as the agent of selection. However, temperature variation is confounded by other related ecological factors, such as rainfall, humidity, food supply and population density.

Laboratory populations maintained at different temperatures provide a means of directly assessing the effects of temperature, or an associated variable, on Drosophila body size (section 1.7). Laboratory thermal selection experiments have shown genetically increased wing length in Drosophila with an evolutionary history of low temperature. This is true for unreplicated (Powell 1974; Cavicchi et al. 1992), and replicated studies (Anderson 1966, 1973) as previously discussed in section 1.7. However, in unreplicated experiments, genetic drift cannot be excluded as a possible cause of population divergence. In studies with replicate populations in each thermal environment, chance divergence can be ruled out as a cause of any difference between thermal lines. Previous long term thermal selection in D. melanogaster (e.g. Cavicchi et al. 1992) did not use replicated populations. The experiments in this thesis therefore represent the first examination of thermal evolution in D. melanogaster under conditions where genetic drift can be ruled out as the cause of any divergence.

Insects such as Drosophila are well suited for studying the cellular basis of both genetic and environmental responses to temperature. Dobzhansky (1929) reported that changes in cell size and number could be easily estimated by examination of

the cuticle of the wing blade, which is secreted by a double layer of cells, each cell forming a tiny trichome. The number of trichomes in a given area of the wing provides a measure of cell density and hence of cell area. Gradients in cell density occur over the surface of the wing, but when counts are made in selected regions and repeated on many wings, the ratio of trichome counts between the regions are found to be highly correlated. If the total wing area is measured, then the cell density in various regions can be used to make different estimates of the total cell number in the wing.

There have been no experimental studies on the cellular basis of the evolutionary response to temperature in laboratory or field populations of Drosophila. The developmental effects of temperature on cell size and number have been reported. Lower temperature during pre-adult development results in increased wing size (Alpatov 1930), which is acheived mainly by changes in cell area, with very much smaller effects on cell number (Alpatov 1930; Robertson 1959a; Delcour and Lints 1966; Masry and Robertson 1979; Cavicchi et al. 1985).

The similarity between the developmental and evolutionary morphological responses to temperature strongly suggest that both are adaptive. The experiment in this chapter re-examined the developmental response to temperature, in terms of its morphological and cellular basis. The nature of the evolutionary response to temperature was also examined, at both the morphological and cellular level. If, indeed, both responses are adaptive then one would expect to see similarities in direction and extent of change.

6.2 Materials and methods

The aim of this experiment was to compare the size of adult flies from the 16.5°C and 25°C selection regimes when reared under standard conditions at both developmental temperatures. Thorax length and wing area were measured and separate estimates of cell size and cell number in the wing were also made. The adults used in this experiment were obtained from vials (twenty per replicate line) which were set up with 32 larvae each, which were used to determine development time (see chapter 3.2.1 for details of materials and methods). The adult flies used in this experiment had two generations of controlled temperature rearing and one generation of controlled density rearing.

A random sample of ten adult flies from each vial was transferred to small Eppendorf tubes containing 70% alcohol. Thorax length was measured using an eyepiece micrometer. Flies were put in a standard position and the measurement taken was from the posterior tip of the scutellum to the base of the most anterior major bristle (see Figure 6.1).

Wings were removed from one male and one female from each vial using fine forceps and dissecting scissors. The wings were then mounted on microscope slides under cover slips using the mounting medium Euparal. When the Euparal had dried, the slides were examined under a compound microscope with a camera lucida attachment. The outline of each wing was drawn and the area subsequently measured using a Summa

Sketch II digitizing pad attached to an Apple MacIntosh SE/30 computer.

The wing surface of Drosophila melanogaster is covered on both sides by tiny trichomes, clearly visible under the microscope. Every epidermal cell in the wing produces a single trichome, enabling cell density and cell area to be estimated for different areas of the wing. The regions 1, 2 and 3 chosen by Dobzhansky (1929) were sampled (see Figure 6.2). The number of trichomes on the dorsal wing surface in each region was recorded with the camera lucida, marking the number of trichomes within a square corresponding to $0.01 mm^2$ of the wing. The reciprocal of the number of trichomes in each measured region gives an estimate of the average cell area in that region. By multiplying the trichome count in each region by the wing area and then dividing this by the area measured ($0.01 mm^2$), three different estimates of the total cell number on the dorsal wing surface were obtained.

6.3 Results

Mean thorax lengths of each sex were calculated for each vial in the experiment, and these mean values used to calculate the mean thorax lengths, which are given for males in Figures 6.3(a) and (b), and for females in Figures 6.4(a) and (b). These results can also be found in Appendix C; Tables C1 and C2. The results of the analyses of variance of these data are given in Tables 6.1 and 6.2. In both sexes the thorax lengths were significantly greater at the lower growth temperature on average by 12.5% and 13.5% for males and females respectively. The main effect

of selection regime was also significant for both sexes, with flies from the 25°C selection regime having, on average, 2% shorter thorax lengths at both 16.5°C and 25°C. There was no significant heterogeneity between the replicate cage lines, as shown by the non significant line within selection effect in Tables 6.1 and 6.2. The effect of growth temperature on thorax length differed between the selection regimes for females, but not for males. This indicates that there was no difference in the extent to which males from the two selection regimes responded to the two growth temperatures, but in females the 16.5°C lines responded more.

The mean wing areas for males reared at 16.5°C and 25°C are given in Figures 6.5(a) and (b) respectively. Figures 6.6(a) and (b) give the mean wing areas for females reared at 16.5°C and 25°C. Appendix C gives the same data in Tables C3 and C4. The results of the analyses of variance in Tables 6.3 and 6.4 show that wing areas in both sexes were significantly larger at the lower developmental temperature. Males and females of the 16.5°C selection regime had larger wing areas than their counterparts from the 25°C selection regime, as indicated by the main effect of selection in Tables 6.3 and 6.4. There was no significant variability in wing area between the replicate cage lines within each selection regime for either sex. There was no significant growth x selection interaction for males or females. (That is, the extent to which males or females from the two selection regimes responded to growth temperature did not differ significantly)

The mean trichome counts at 16.5°C from regions one, two and three are given in Figures 6.7(a) to 6.12(a) for both sexes.

Figures 6.7(b) through 6.12(b) show the trichome counts from all three regions for males and females at 25°C. All this data is given in Appendix C Tables C5 to C10. The results of the analyses of variance are given in Tables 6.5 to 6.10. In all three regions in both sexes, the effect of growth temperature was highly significant: at 16.5°C the trichome count was much lower than at 25°C for flies from both selection regimes. (For 16.5°C selected males the reduction in trichome count was 19.5%, 11.1% and 22.1% for regions 1, 2 and 3 compared with 23.8%, 15.6% and 25.4% for 25°C selected males. Trichome counts for 16.5°C selected females were reduced by 20.6%, 23.8% and 22.6% compared with 12.2%, 27.1% and 26.5% for 25°C selected females, for regions 1, 2 and 3 respectively.) There was a significant effect of selection for both males and females in each of regions one, two and three. Flies from the 25°C selection regime had greater trichome counts than flies from the 16.5°C selection regime. (This averaged 5.6% and 10.2% for males and females at 16.5°C and 10.1% and 10.3% for males and females at 25°C.) There was significant heterogeneity in trichome count between replicate cage lines only for region one in females and region two in males, as shown in Tables 6.6 and 6.7 respectively. There was a significant growth x selection interaction for males in regions one and for males and females in region three. In all these cases trichome count increased to a greater extent in the 25°C selected lines, compared to the 16.5°C selected lines, when they developed at 25°C than at 16.5°C. As trichome count reflects the average cell area in each region measured, one can conclude that development at the lower temperature produced larger cell areas in adults from both

selection regimes. Furthermore, at both developmental temperatures adults from the 16.5°C selection regime had significantly larger cell areas than did 25°C selected adults and this was repeated in all three regions measured.

The estimates of mean total cell numbers on each dorsal wing surface, based on the trichome counts for regions 1, 2 and 3 in both sexes are given in Figures 6.13(a) and (b) to 6.18(a) and (b), with the results of the analyses of variance in Tables 6.11 to 6.16. The data from Figures 6.13 to 6.18 are given in Appendix C; Tables C11 to C16. The effect of growth temperature on cell number was not significant for regions 2 and 3 for females, but was significant in all other cases; females for region 1, and males for regions 1, 2 and 3. Total cell number was generally greater at the lower developmental temperature, but the converse was found in two cases (25°C selected females in areas 2 and 3). There was a significant main effect of selection regime in only two cases; females in regions 1 and 3, with 25°C selected females having greater cell numbers than 16.5°C selected females at both developmental temperatures. Only regions 1 and 2 showed any significant heterogeneity between replicate lines within selection regimes. There were no significant growth X selection interactions for either sex, in any region.

6.4 Discussion

Thorax length and wing area were both significantly larger at the lower developmental temperature. Evolution at low temperature also resulted in increased thorax and wing length, as shown by the consistent differences between the 16.5°C and 25°C thermal selection lines. The similarity between these developmental and evolutionary morphological responses to temperature suggest that both are adaptive, with the developmental response being a form of adaptive phenotypic plasticity (Stearns 1989; Kirkpatrick and Lofsvold 1992). The results confirm and extend previous work on several Drosophila species (see section 6.1 and Chapter 1).

As mentioned in the introduction to this chapter, several previous studies have reported that developmental temperature affects mainly cell size in Drosophila (Alpatov 1930; Robertson 1959a; Delcour and Lints 1966; Masry and Robertson 1979; Cavicchi et al. 1985). This is in contrast to the response to artificial selection for body size and the genetic variance for body size segregating in natural populations, both of which involve mainly changes in cell number (Zarapkin 1934; Robertson 1959a, 1959b). Recent work has shown that artificial selection for increased body size involves an increase in cell number (with no effect on cell size), while artificial selection for decreased body size is predominantly a function of reduced cell size (Langelan et al, unpublished data). In the experiment reported here, both the evolutionary and developmental thermal responses in wing area resulted mainly from an increase in cell size at the lower temperature. The evolutionary

effects of temperature have not previously been examined in terms of their cellular basis and, once again their similarity to the developmental effects suggests that the developmental response to temperature is adaptive.

It is puzzling why large larvae or adults should be at a greater advantage at lower temperature. Some studies have reported that large flies have greater fitness at all temperatures (eg. Cavicchi et al. 1985). However, experiments reported in this thesis have shown that genetically large flies may have longer developmental periods (chapter 3); lower larval competitive ability (chapter 5); lower lifetime reproductive success and shorter lifespans (chapter 7) compared to genetically small flies. Artificial selection for increased body size can result in a correlated increase in pre-adult mortality (Partridge and Fowler, 1993). It is reasonable to assume that body size is only one of a set of co-adapted characters which have responded to thermal selection. Although 16.5°C selected flies were larger than the 25°C selected flies when both were reared at 25°C, they took longer to develop, and showed lower larval viability and lower larval competitive ability (Chapters 3 and 5). The converse was true when both selected lines were reared at 16.5°C. These fitness differences could be partly attributable to genetic differences other than those related to cell size.

Thermal selection leads to a decrease in cell size and body size at high temperature. This suggests that small cell and/or body size is adaptive at high temperature. However, the situation is more complex in the wild. While studying thermal acclimation in a number of Drosophila species, Levins (1969) found that D. melanogaster from hot coastal locations in Puerto Rico were

genetically larger than flies from cool upland locations. If temperature were the only factor then this result is the opposite of that predicted by a thermal selection hypothesis. Levins (1969) proposed that the difference between his results and others (e.g. Tantaway and Mallah 1961) was a consequence of different humidity regimes in the transects examined. For example, in Puerto Rico, cool areas are also wet, and hot areas are dry, while the opposite situation occurs for the transect in the Middle East studied by Tantaway and Mallah (1961). In hot, dry habitats higher temperature of development would result in shorter development times and smaller body size, whereas the desiccating conditions would favour selection for larger adult body size (Levins 1969). Adaptation for desiccation resistance appears to be an important component of selection on adult body size in Drosophila, which is confounded with temperature adaptation (Levins 1969; Ringo and Wood 1984; Hoffmann 1990; 1991; Thomas and Barker 1993).

It is unclear why cell size rather than cell number should evolve in response to thermal selection. If the primary target of thermal selection were body size, it could simply be that change in cell size is the easiest way to achieve this. However, artificial selection for increased body size has been shown to involve mainly changes in cell number, with little effect on cell size (Zarapkin 1934; Robertson 1959a). Alternatively selection could be directly on cell size, with body size changing as a consequence but being selectively nuetral. All body parts of Drosophila become larger at lower temperature, but this has not yet been demonstrated to be due to changes in cell size except in the wing blade. In the Drosophila wing, the epidermal cells are very flattened at the time of cuticle

secretion, so estimation of cell area by trichome counts may not be a reliable indicator of cell size (Kuo and Larsen 1987). To determine if different regions of epidermis, which develop from imaginal discs, respond similarly to temperature it would be neccessary to measure cell density in defined regions of epidermis. It would also be possible to test whether cell size is adaptive by performing artificial selection experiments on cell size per se (i.e. selecting upwards on cell size, and then measuring the relative fitness of generated lines at different temperatures).

Geographical size clines in Drosophila have been well documented (section 1.4 - 1.7), and have been shown to be partly genetic. However, the cellular basis for such size clines has not been studied. In order to establish whether findings in the laboratory, such as those reported in this chapter, extend to the field, it would be neccessary to collect different geographical populations and rear them at a common temperature. It would also be interesting to see whether temperature has indeed been the principal selective agent in the wild. This could be done by looking at the thermal adaptation of various field collected stocks, and examining development times, larval viabilities, adult fertility and longevity at different temperatures. Any correlation of increased fitness at low temperatures with large cell size and body size (or the converse at high temperature) in geographical populations reared under controlled laboratory conditions would provide compelling evidence for the importance of temperature on the evolution of body size in the wild and laboratory.

With the imminent prospect of rapid global warming (IPCC 1992), changing many other climatic and ecological variables, such as rainfall, seasonal fluctuations and food supply, further work is essential to establish a link between laboratory and field work on the direct effects of temperature on Drosophila body size and life history. We are still largely ignorant of a large area of thermal biology. Future work on wild collected populations may help in our understanding of the thermal effects on cell-size, body-size and other life history characters in Drosophila and ectotherms in general.

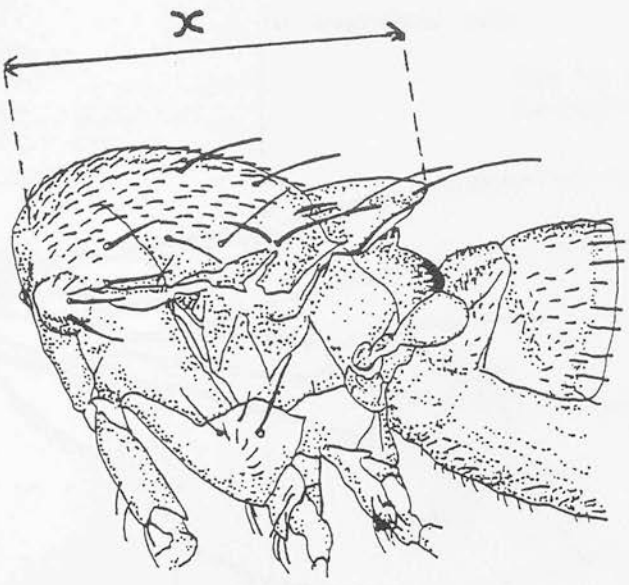


Figure 6.1 Lateral view of the thorax of Drosophila melanogaster, showing the dimension used to score thorax length.

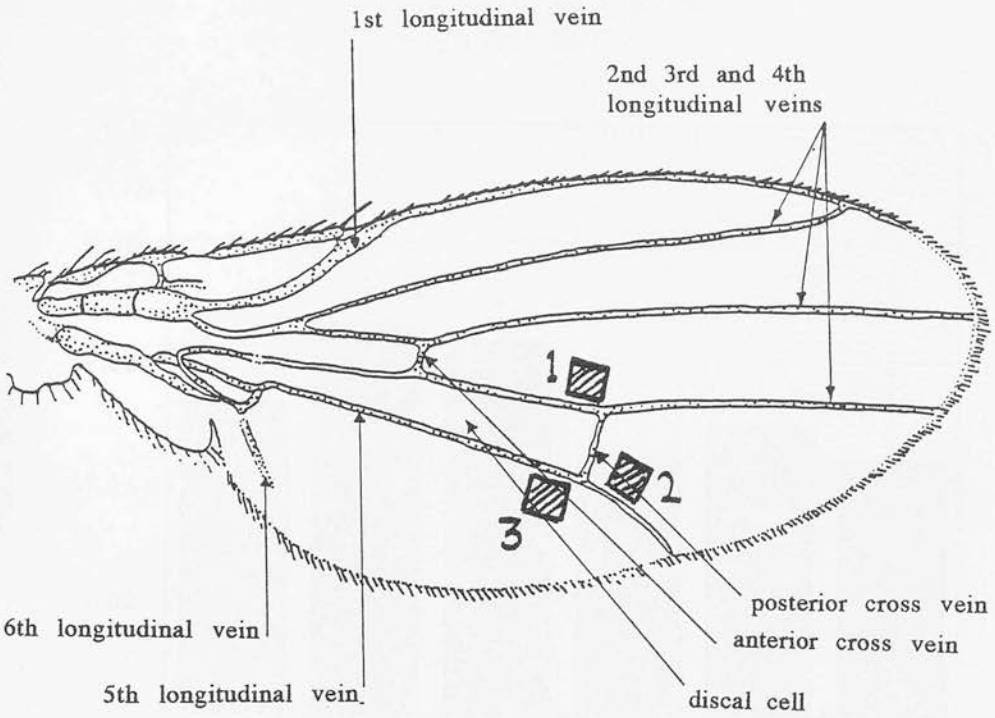
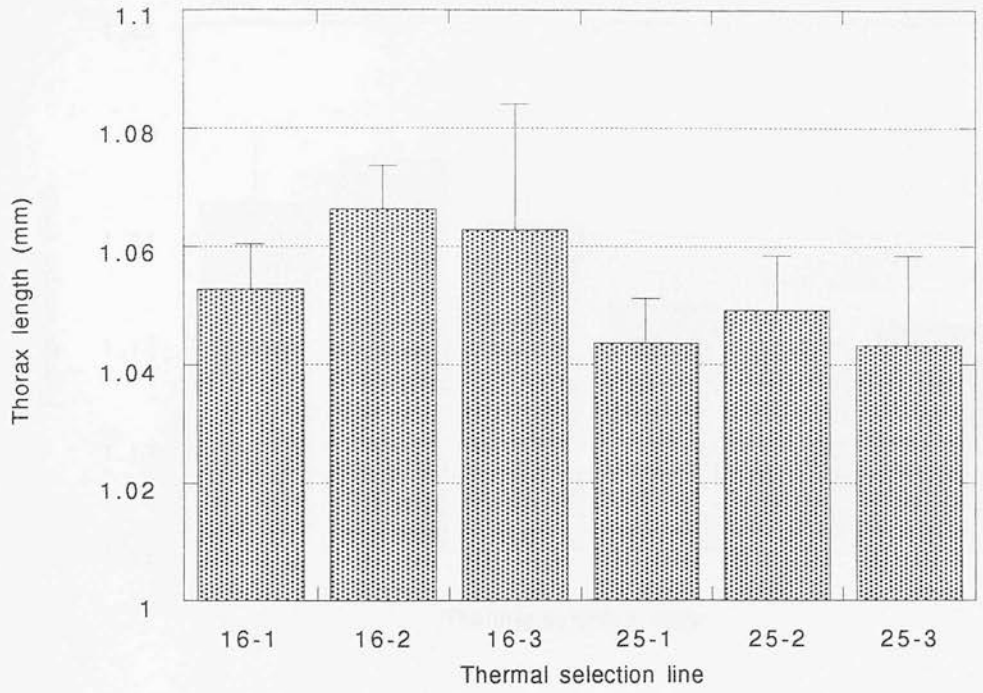


Figure 6.2 Wing of Drosophila melanogaster, showing the position of the regions 1, 2 and 3 in which the size of the cells has been studied.

Figure 6.3(a) Mean thorax lengths for males reared at 16.5°C
(+/- 95% confidence intervals)



(b) Mean thorax lengths for males reared at 25°C.
(+/- 95% confidence intervals)

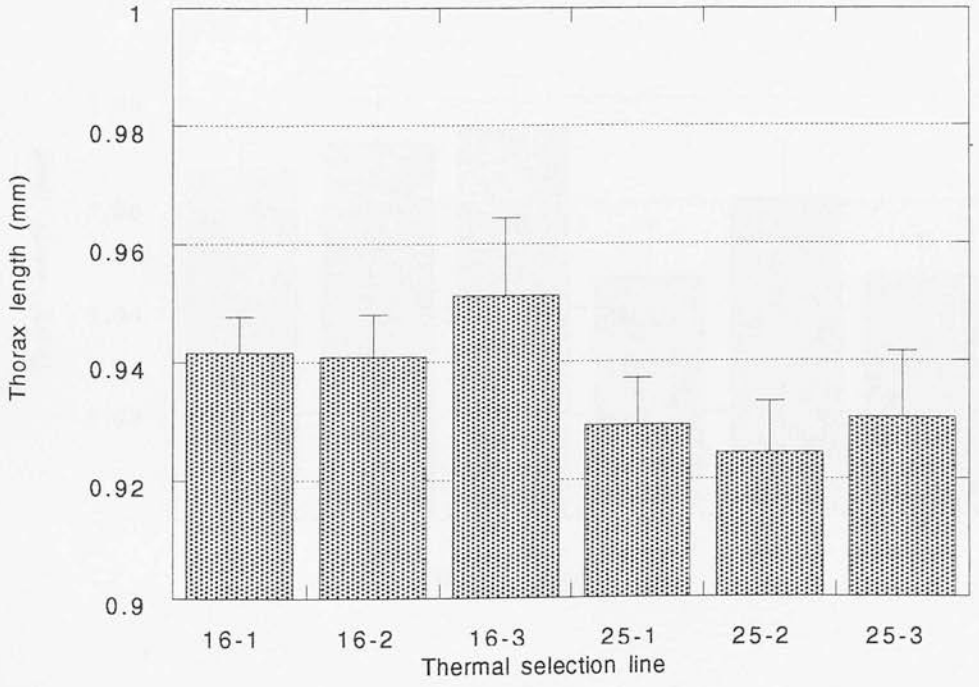
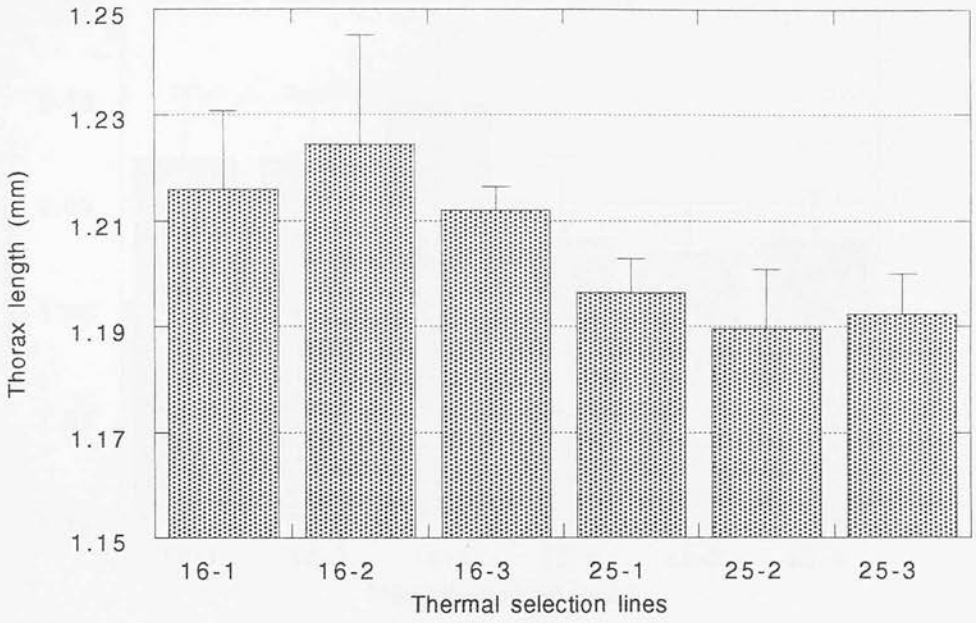


Figure 6.4(a) Mean thorax lengths for females at 16.5°C
(+/- 95% confidence intervals)



(b) Mean thorax lengths for females at 25°C
(+/- 95% confidence intervals)

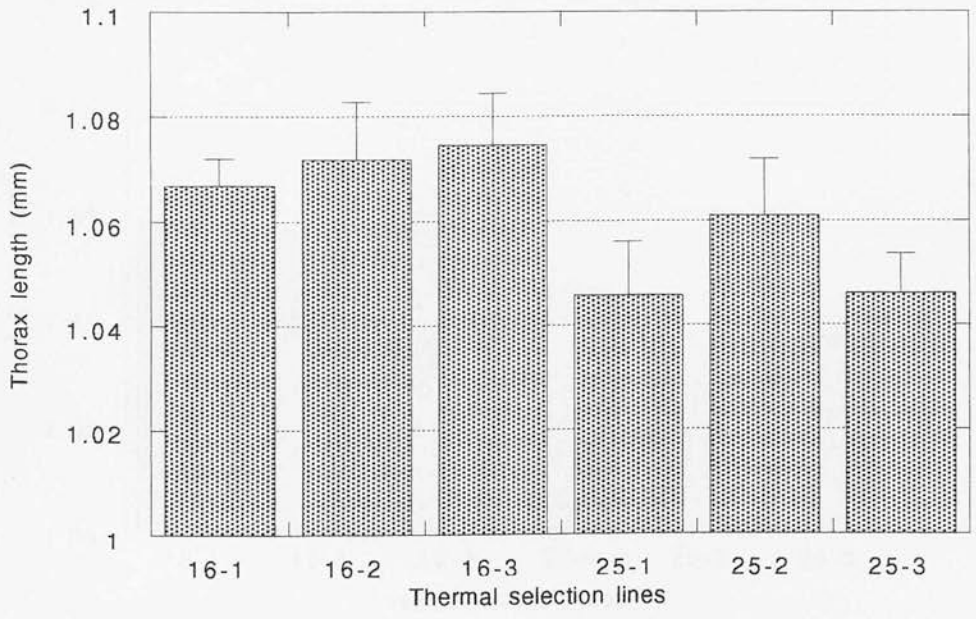
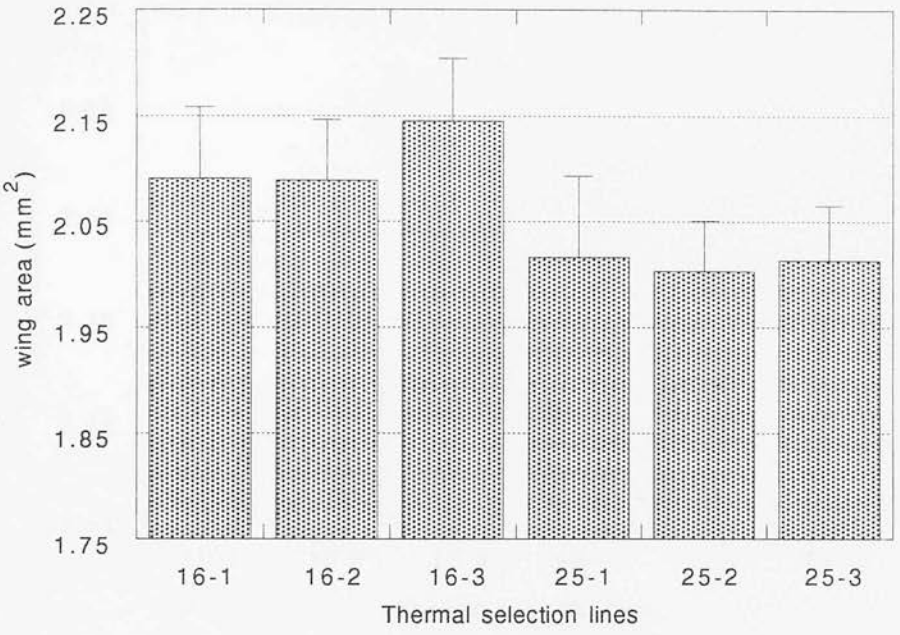


Figure 6.5(a) Mean wing areas for males at 16.5°C
(+/- 95% confidence intervals)



(b) Mean wing areas for males at 25°C
(+/- 95% confidence intervals)

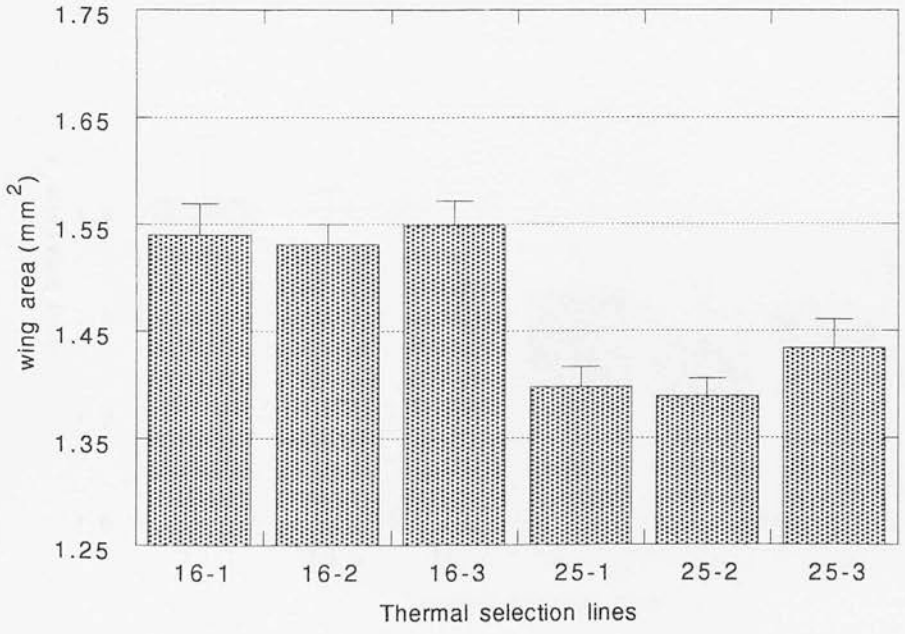
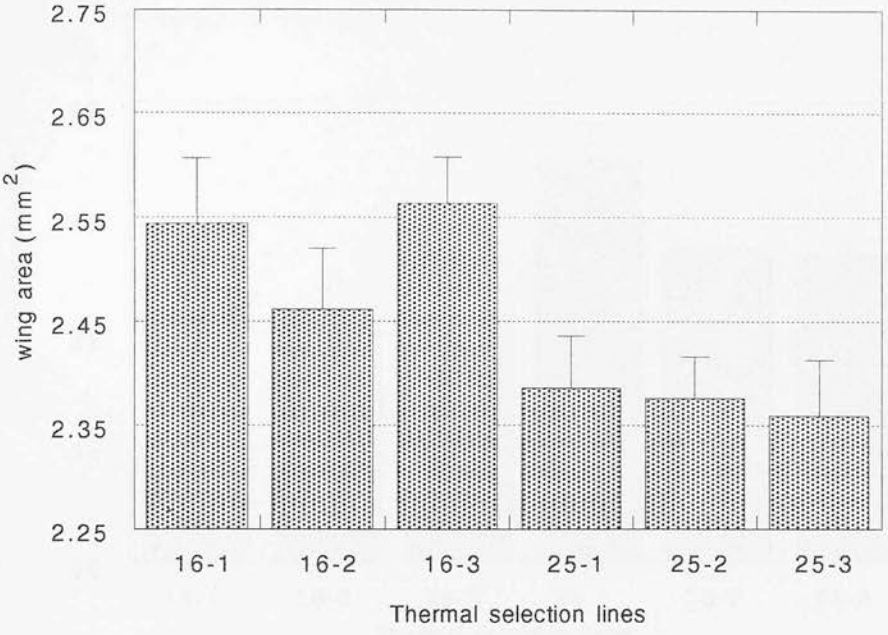


Figure 6.6(a) Mean wing areas for females at 16.5°C
(+/- 95% confidence intervals)



(b) Mean wing areas for females at 25°C
(+/- 95% confidence intervals)

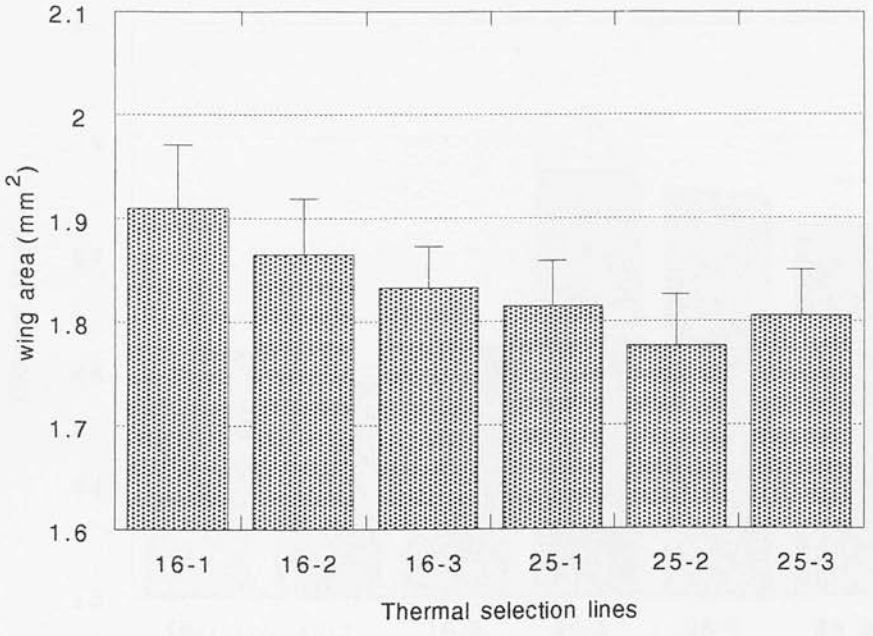
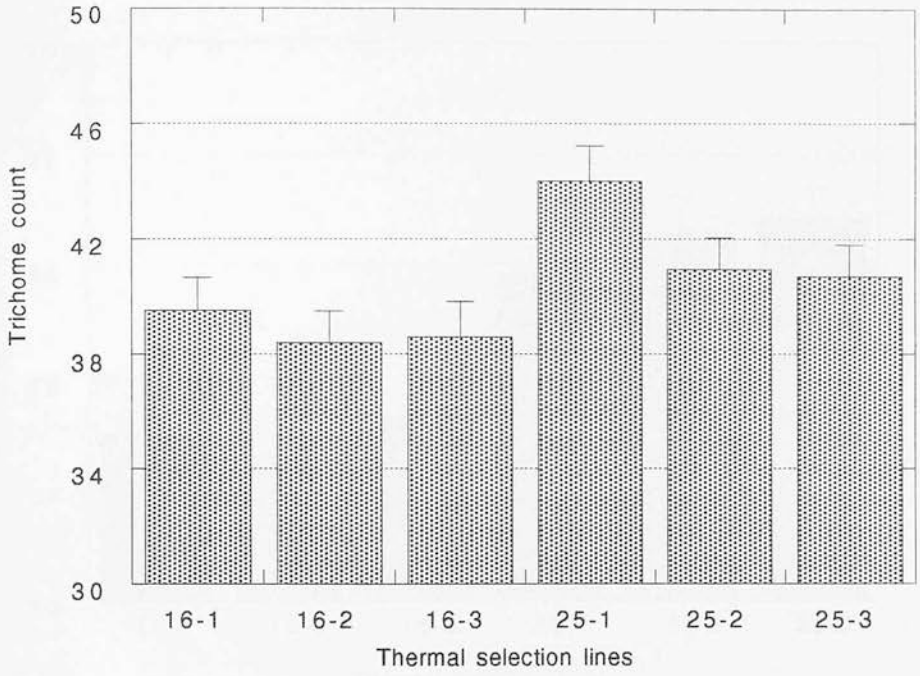


Figure 6.7(a) Mean trichome counts from region 1 for males reared at 16.5°C. (+/- 95% confidence intervals).



(b) Mean trichome counts from region 1 for males at 25°C (+/- 95% confidence intervals)

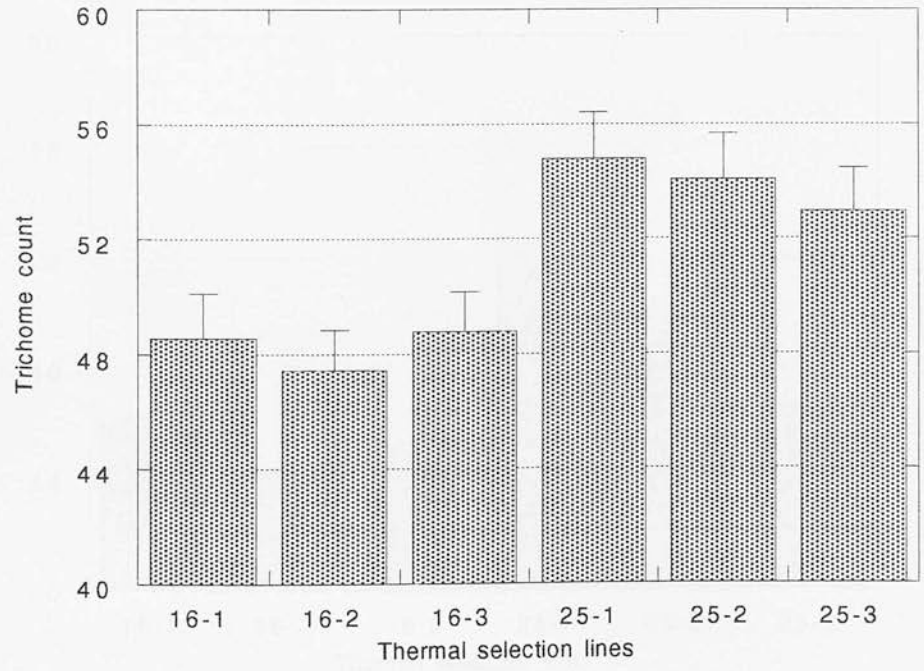
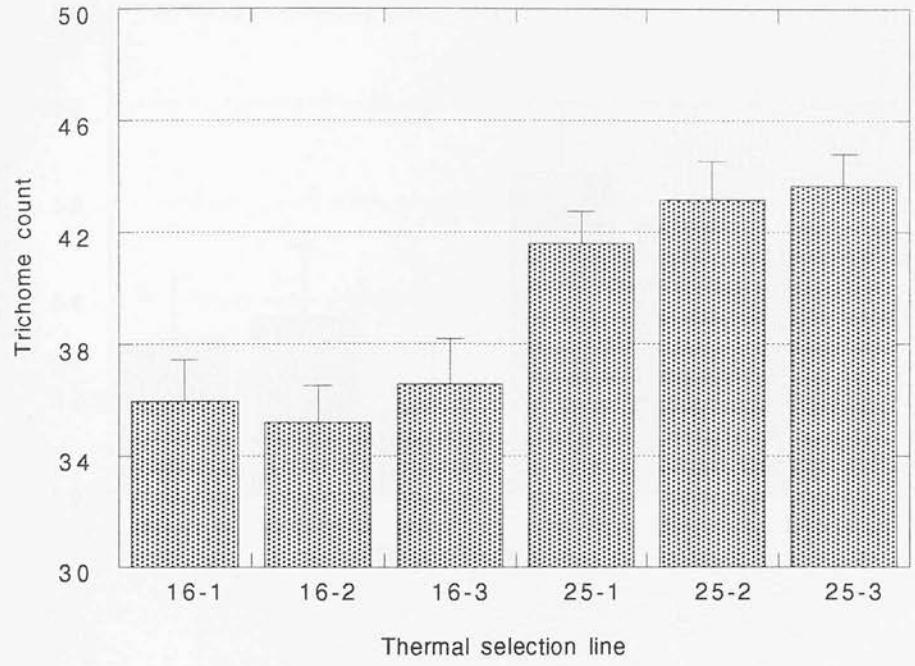


Figure 6.8(a) Mean trichome counts from region 1 for females at 16.5°
(+/- 95% confidence intervals)



(b) Mean trichome counts from region 1 for females at 25°C
(+/- 95% confidence intervals)

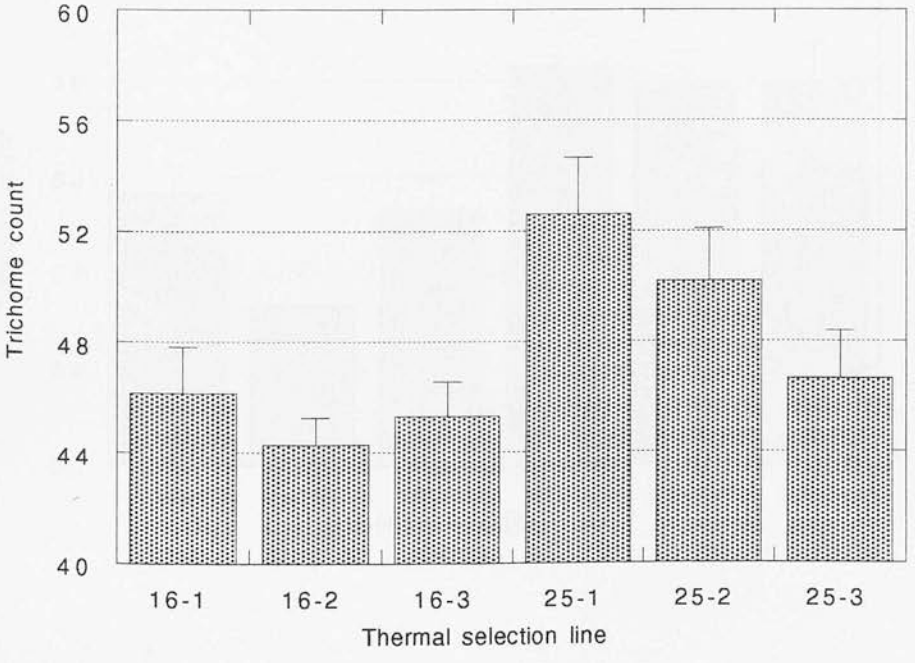
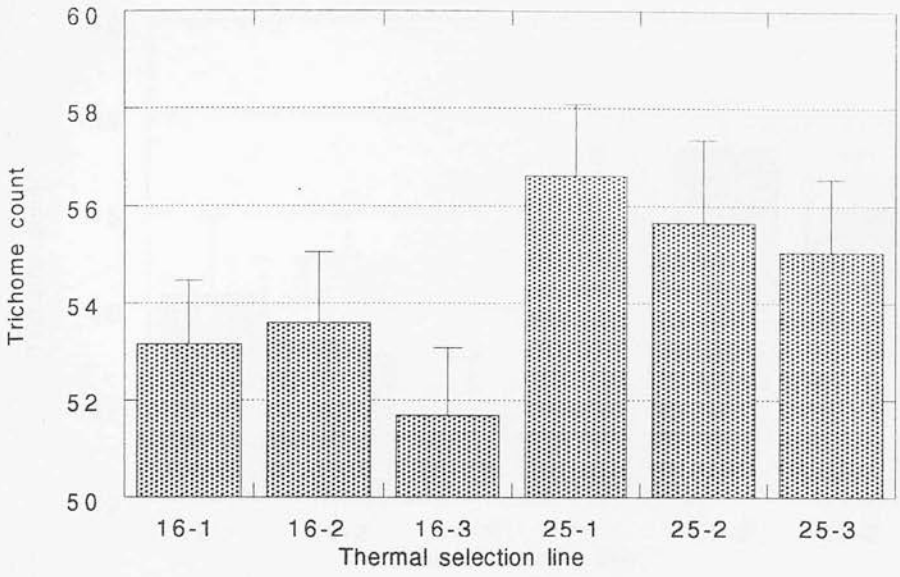


Figure 6.9(a) Mean trichome counts from region 2 for males at 16.5°C (+/- 95% confidence intervals)



(b) Mean trichome counts from region 2 for males at 25°C (+/- 95% confidence intervals)

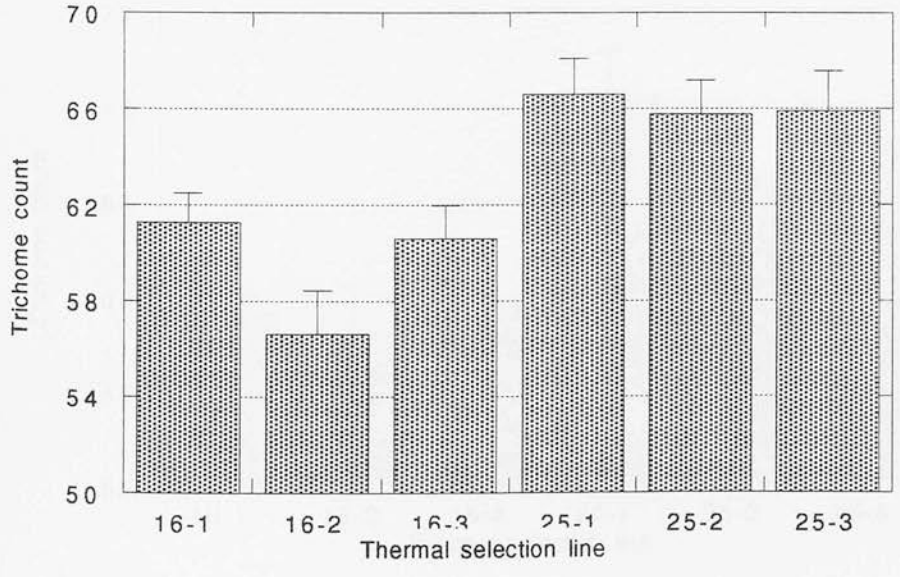
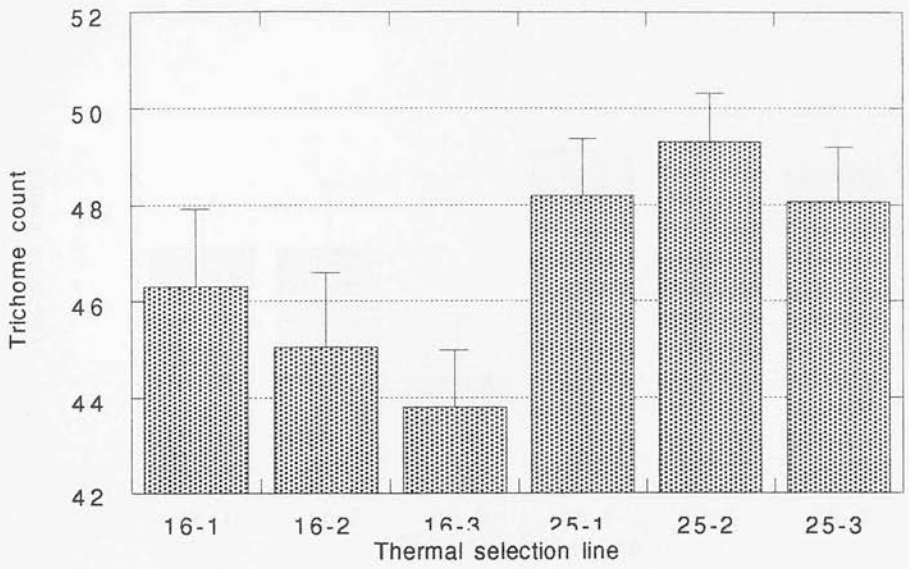


Figure 6.10(a) Mean trichome counts from region 2 for females at 16.5°C (+/- 95% confidence intervals)



(b) Mean trichome counts from region 2 for females at 25°C (+/- 95% confidence intervals)

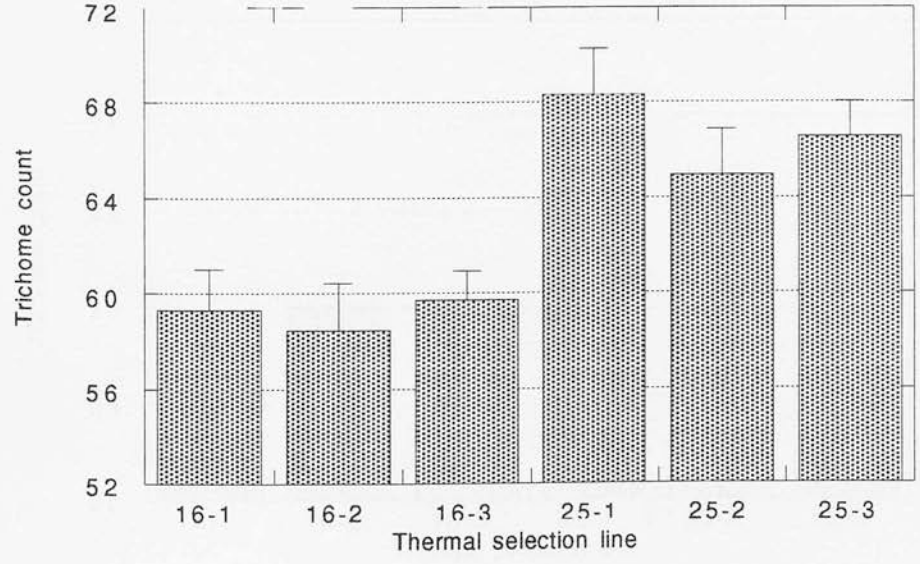
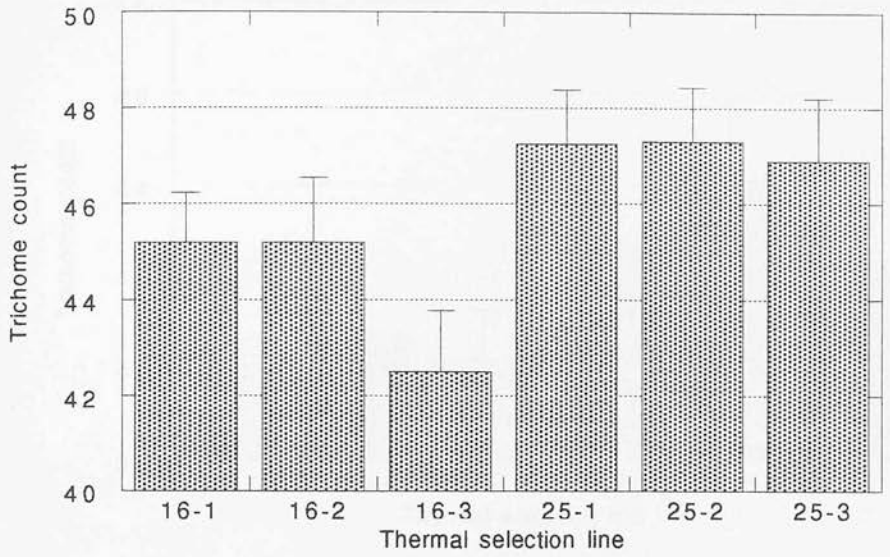


Figure 6.11(a) Mean trichome counts from region 3 for males at 16.5°C (+/- 95% confidence intervals)



(b) Mean trichome counts from region 3 for males at 25°C (+/- 95% confidence intervals)

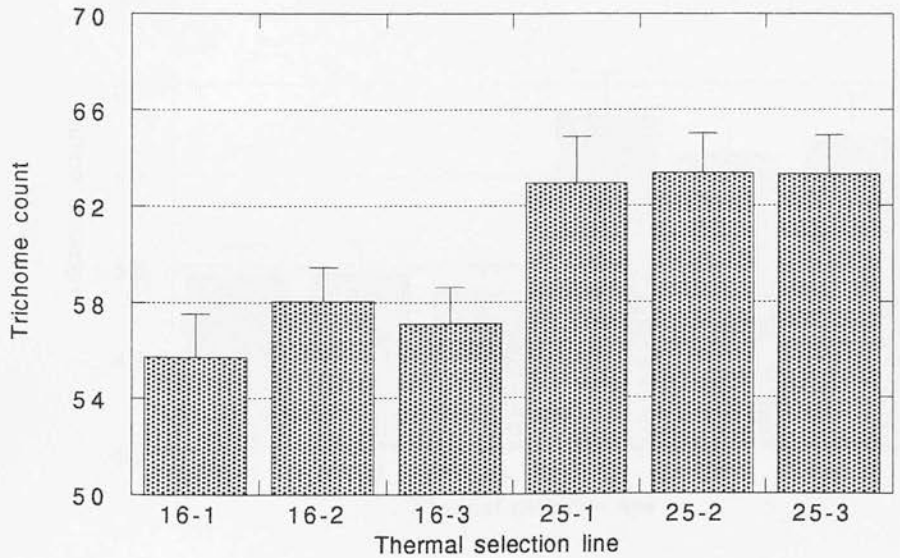
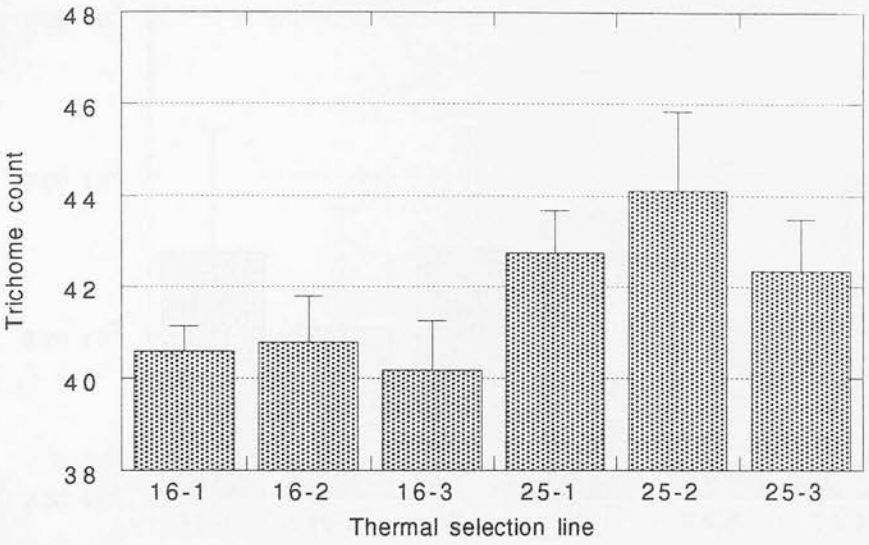


Figure 6.12(a) Mean trichome counts from region 3 for females at 16.5°C (+/- 95% confidence intervals)



(b) Mean trichome counts from region 3 for females at 25°C (+/- 95% confidence intervals)

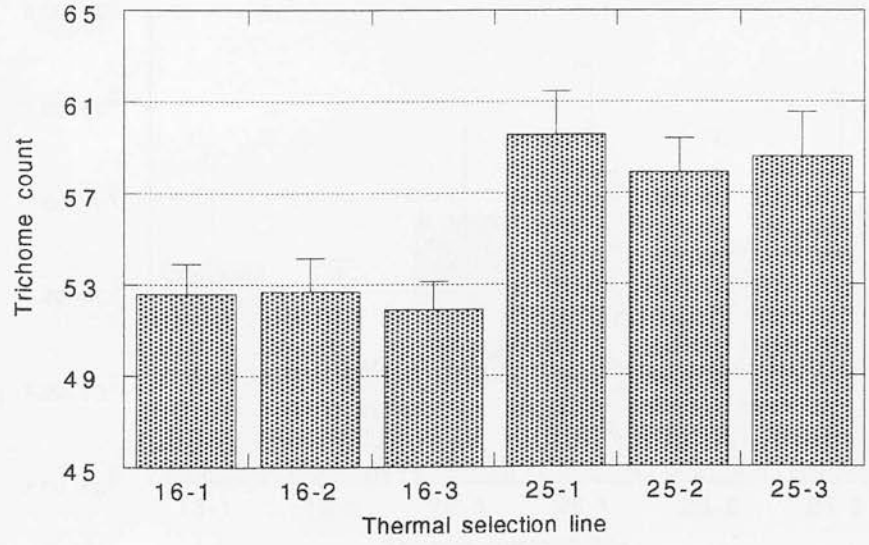
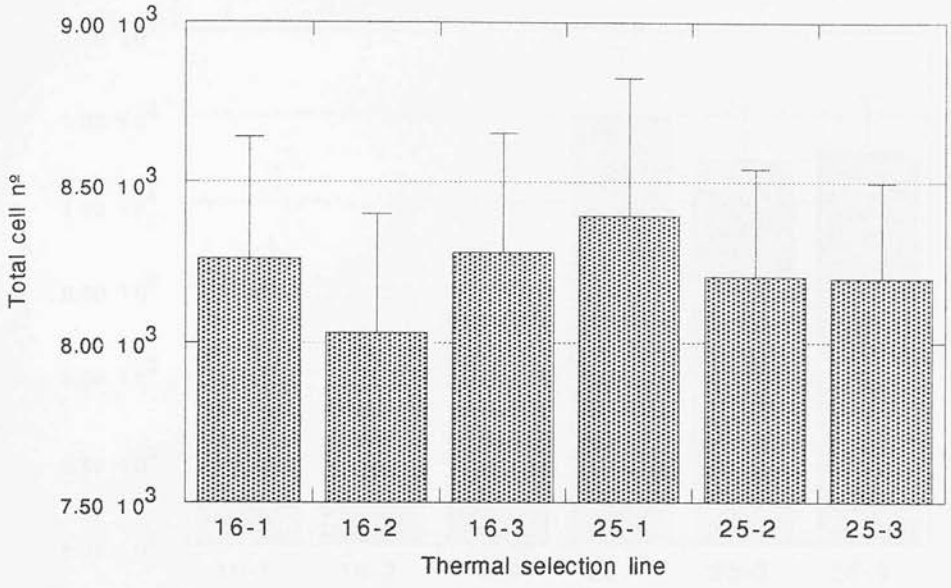


Figure 6.13(a) Mean total dorsal cell nº estimates from region 1- males at 16.5°C (+/- 95% confidence intervals)



(b) Mean total dorsal cell nº estimates from region 1- males at 25°C (+/- 95% confidence intervals)

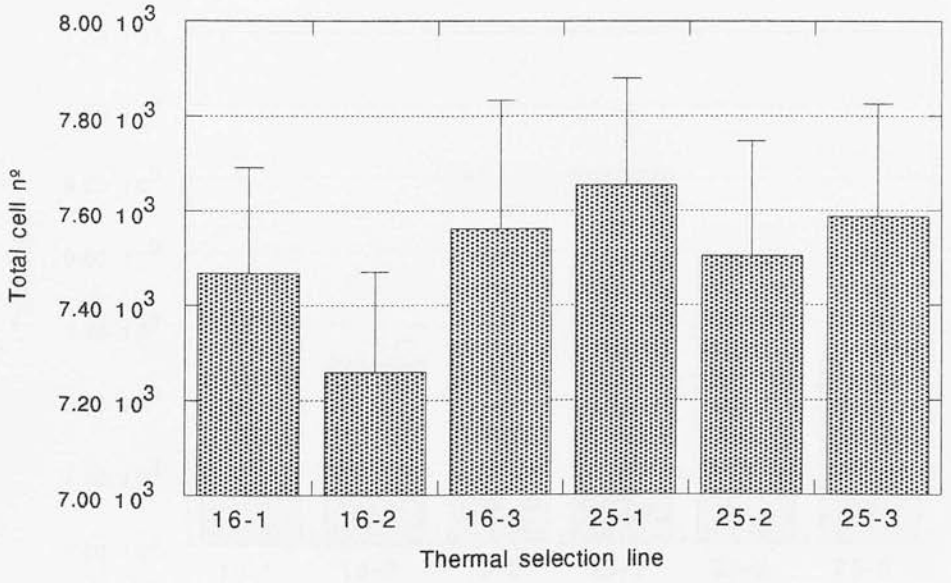
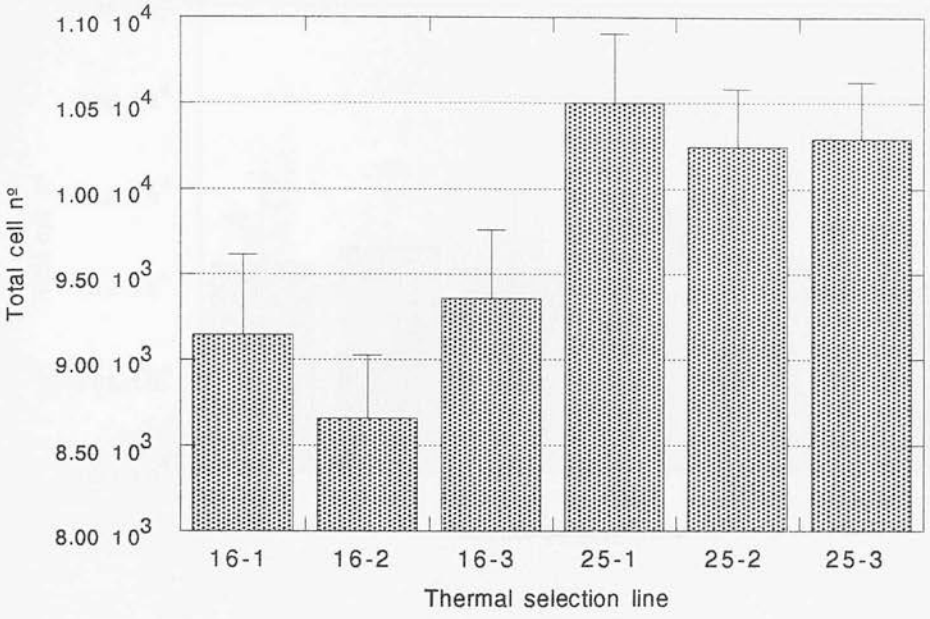


Figure 6.14(a) Mean total dorsal cell nº estimates from region 1- females at 16.5°C (+/- 95% confidence intervals)



(b) Mean total dorsal cell nº estimates from region 1- females at 25°C (+/- 95% confidence intervals)

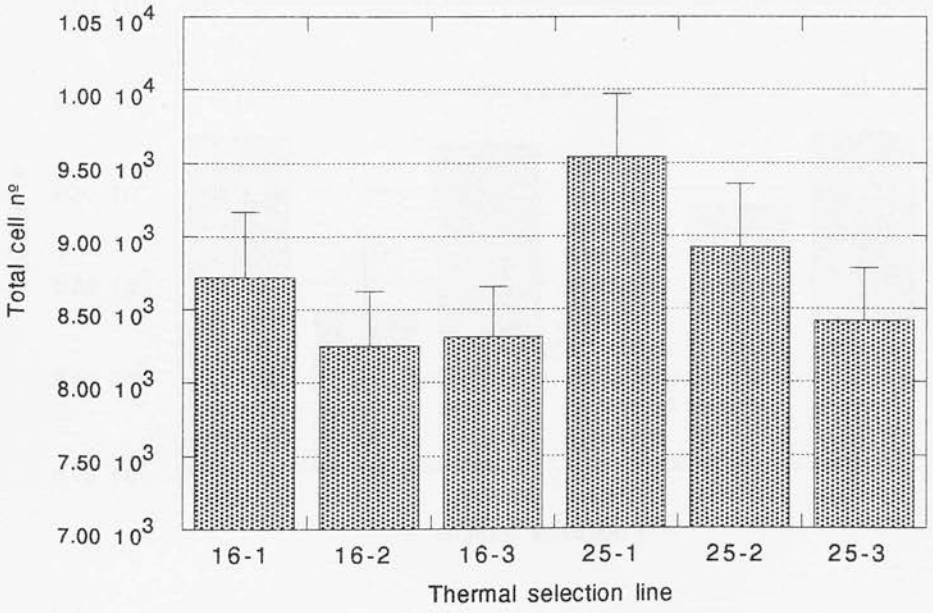
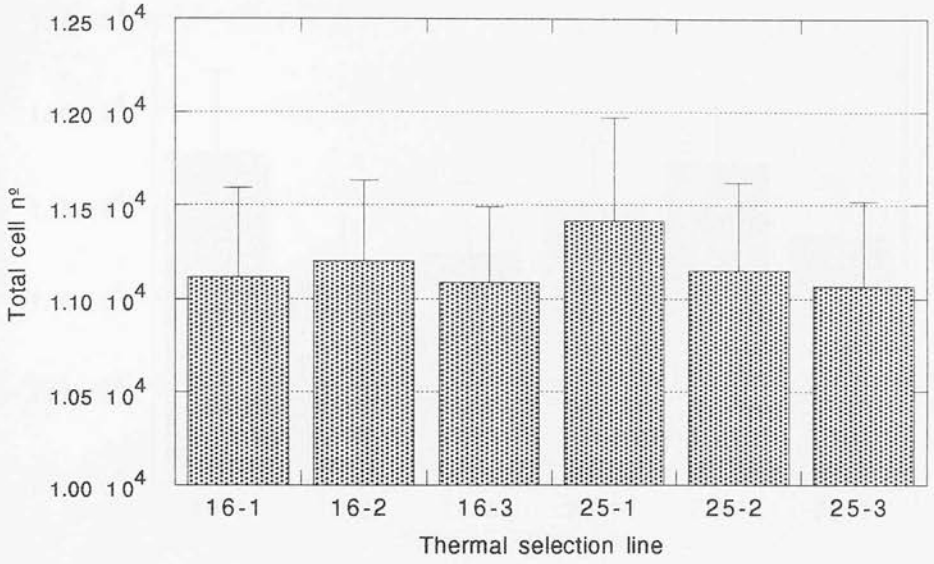


Figure 6.15(a) Mean total dorsal cell nº estimates from region 2 - males at 16.5°C (+/- 95% confidence intervals)



(b) Mean total dorsal cell nº estimates from region 2 - males at 25°C (+/- 95% confidence intervals)

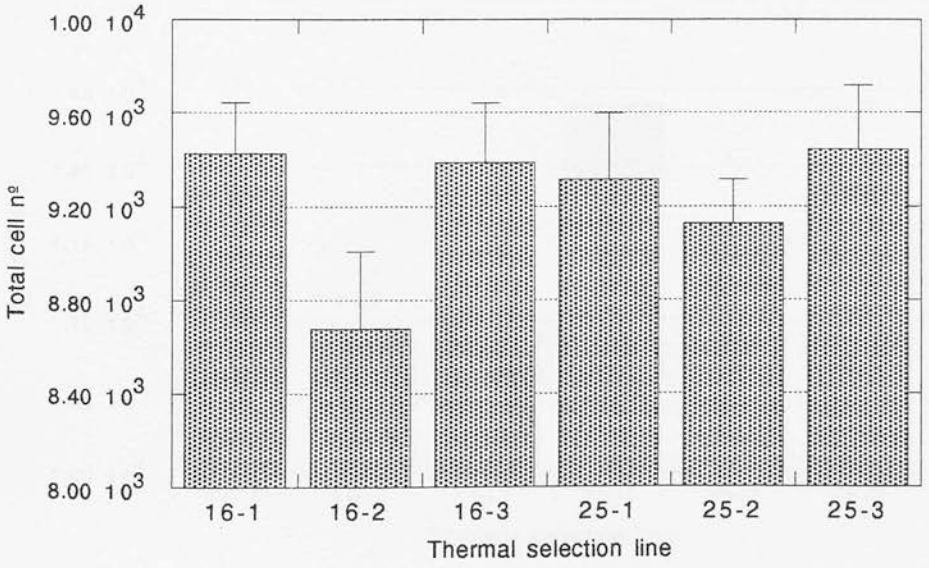
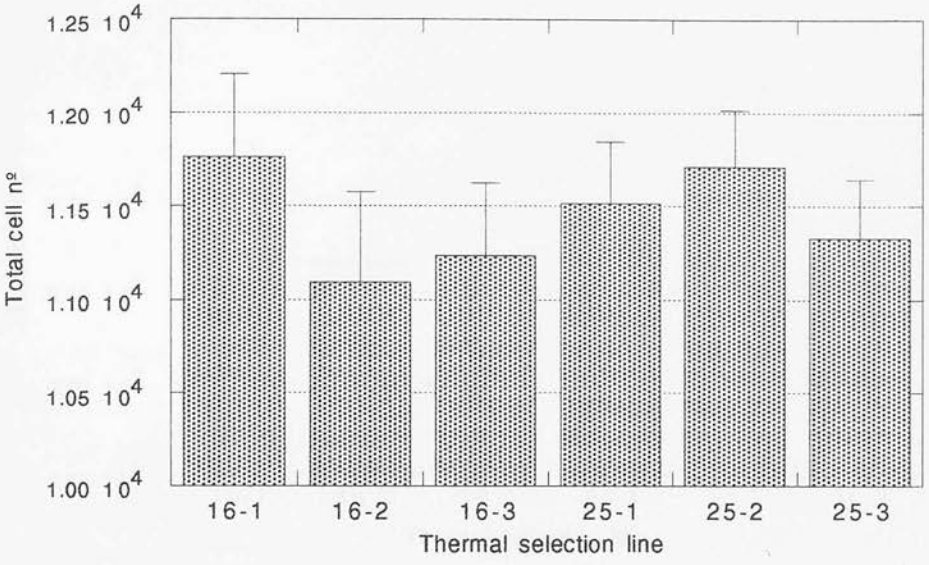


Figure 6.16(a) Mean total dorsal cell nº estimates from region 2 - females at 16.5°C (+/- 95% confidence intervals)



(b) Mean total dorsal cell nº estimates from region 2 - females at 25°C (+/- 95% confidence intervals)

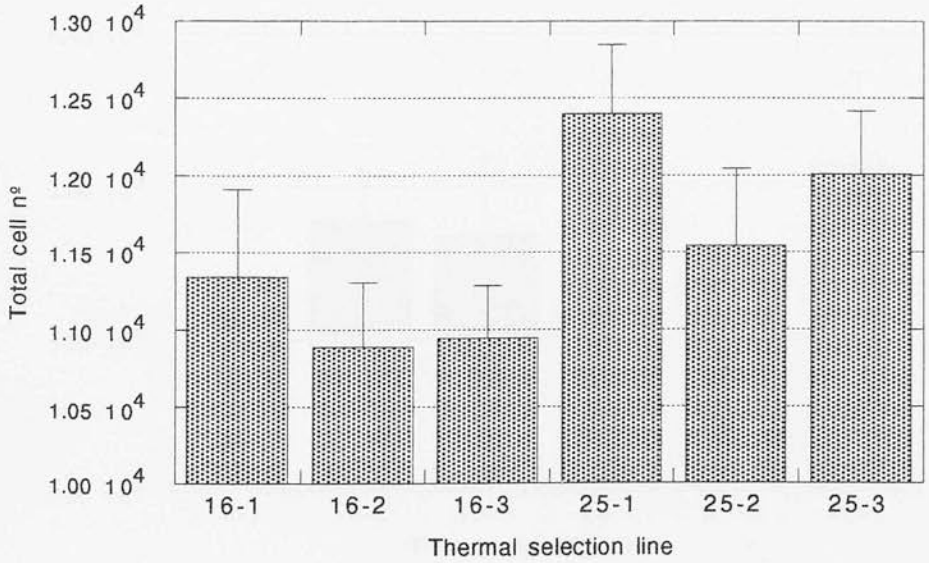
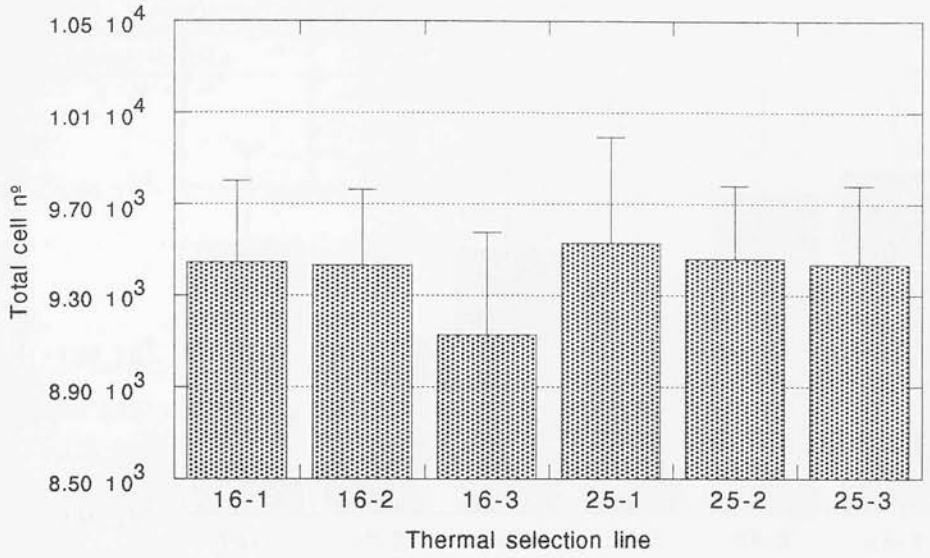


Figure 6.17(a) Mean total dorsal cell nº estimates from region 3 - males at 16.5°C (+/- 95% confidence intervals)



(b) Mean total dorsal cell nº estimates from region 3 -males at 25°C (+/- 95% confidence intervals)

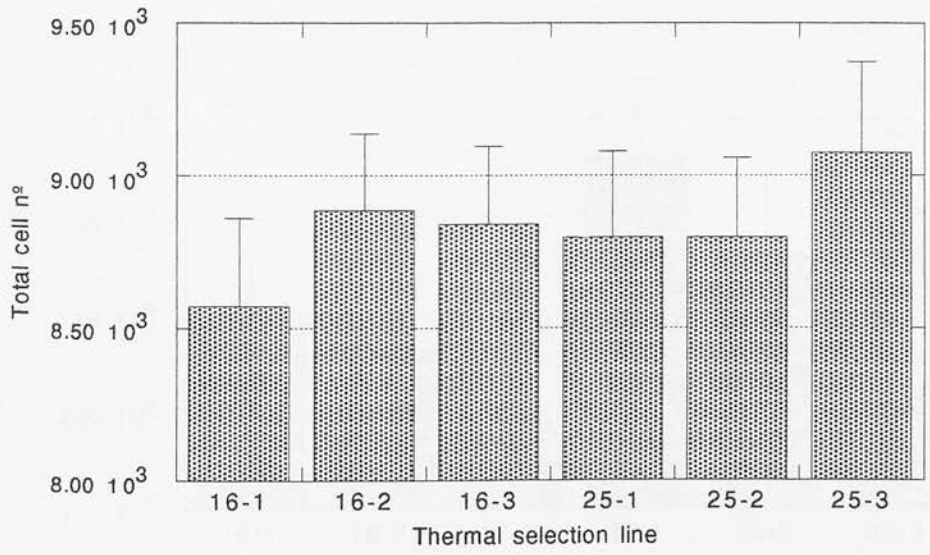
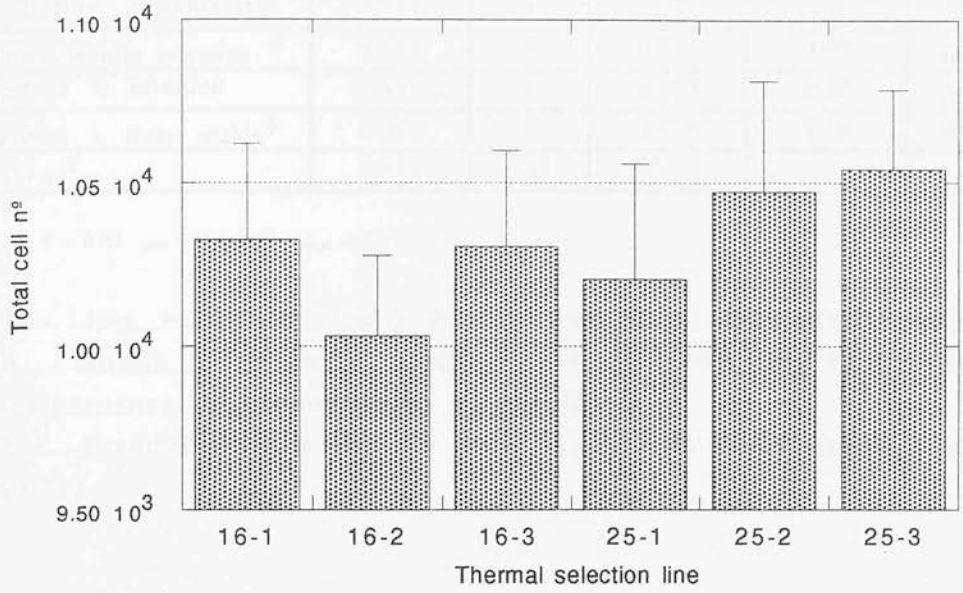


Figure 6.18(a) Mean total dorsal cell number estimates from region 3 - females at 16.5°C (+/- 95% confidence intervals)



(b) Mean total dorsal cell nº estimates fro region 3 - females at 25°C (+/- 95% confidence intervals)

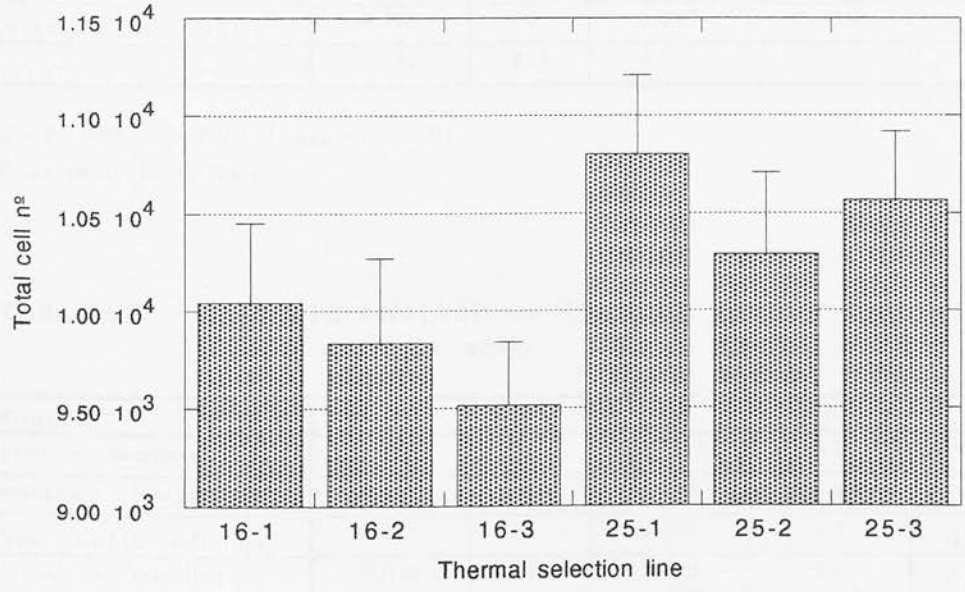


TABLE 6.1 NESTED ANALYSIS OF VARIANCE
Thorax lengths for males

source	ss	df	ms	F	P
growth temperature	599.29	1	599.29	785.44	***
selection temperature	23.41	1	23.41	149	***
lines within selection [A]	0.628	4	0.157	0.98	n.s.
growth x selection	0.153	1	0.153	0.20	n.s.
growth x lines within [B]	0.305	4	0.076	4.76	***
error [C]	36.56	228	0.160		

* = P < 0.05 ** = P < 0.01 *** = P < 0.001

A.....Lines within selection is the error term for selection temperature.
B.....Growth x lines within selection is the error term for both growth temperature and for growth x selection.
C.....Error is the error term for lines within selection and growth x lines within.

TABLE 6.2 NESTED ANALYSIS OF VARIANCE
Thorax lengths for females

source	ss	df	ms	F	P
growth temperature	854.28	1	854.28	8852	***
selection temperature	29.82	1	29.82	53.7	**
lines within selection [A]	2.219	4	0.555	2.14	n.s.
growth x selection	1.873	1	1.873	19.4	*
growth x lines within [B]	0.386	4	0.097	0.359	n.s.
error [C]	61.30	228	0.269		

* = P < 0.05 ** = P < 0.01 *** = P < 0.001
Error terms as in Table 6.1

TABLE 6.3 NESTED ANALYSIS OF VARIANCE
Wing areas for males

source	ss	df	ms	F	P
growth temperature	20.610	1	20.610	4122	* * *
selection temperature	0.799	1	0.799	66.58	* *
lines within selection [A]	0.047	4	0.012	1.2	n.s.
growth x selection	0.019	1	0.019	3.8	n.s.
growth x lines within [B]	0.021	4	0.005	0.5	n.s.
error [C]	2.192	228	0.010		

* = P < 0.05 ** = P < 0.01 *** = P < 0.001
Error terms as in Table 6.1

TABLE 6.4 NESTED ANALYSIS OF VARIANCE
Wing areas for females

source	ss	df	ms	F	P
growth temperature	22.632	1	22.632	838.22	***
selection temperature	0.726	1	0.726	31.56	**
lines within selection[A]	0.093	4	0.023	1.92	n.s.
growth x selection	0.094	1	0.094	3.48	n.s.
growth x lines within[B]	0.106	4	0.027	2.25	n.s.
error[C]	2.688	228	0.012		

* = P < 0.05 ** = P < 0.01 *** = P < 0.001
Error terms as in Table 6.1

TABLE 6.5 NESTED ANALYSIS OF VARIANCE
Trichome counts - region 1 - males

source	ss	df	ms	F	P
growth temperature	7437.067	1	7437.067	1998.674	***
selection temperature	944.067	1	944.067	60.892	**
lines within selection[A]	62.017	4	15.504	1.898	n.s.
growth x selection	173.400	1	173.400	46.600	**
growth x lines within[B]	14.883	4	3.721	0.456	n.s.
error[C]	1862.300	228	8.168		

* = P < 0.05 ** = P < 0.01 *** = P < 0.001
Error terms as in Table 6.1

TABLE 6.6 NESTED ANALYSIS OF VARIANCE
Trichome counts - region 1 - females

source	ss	df	ms	F	P
growth temperature	3542.017	1	3542.017	82.340	***
selection temperature	2331.267	1	2331.267	39.703	**
lines within selection[A]	234.867	4	58.717	5.562	***
growth x selection	129.067	1	129.067	3.000	n.s.
growth x lines within[B]	172.067	4	42.017	4.075	**
error[C]	2406.700	228	10.556		

* = P < 0.05 ** = P < 0.01 *** = P < 0.001
Error terms as in Table 6.1

TABLE 6.7 NESTED ANALYSIS OF VARIANCE
Trichome counts - region 2 -males

source	ss	df	ms	F	P
growth temperature	4352.017	1	4352.017	84.232	* * *
selection temperature	1353.750	1	1353.750	46.548	* *
lines within selection[A]	116.333	4	29.083	2.871	*
growth x selection	201.667	1	201.667	3.903	n.s.
growth x lines within[B]	206.667	4	51.667	5.101	* * *
error[C]	2309.300	228	10129		

* = P < 0.05 ** = P < 0.01 *** = P < 0.001
Error terms as in Table 6.1

TABLE 6.8 NESTED ANALYSIS OF VARIANCE
Trichome counts - region 2 - females

source	ss	df	ms	F	P
growth temperature	15520.417	1	15520.417	416.611	* * *
selection temperature	1804.017	1	1804.017	114.152	* * *
lines within selection[A]	63.017	4	15.754	1.392	n.s.
growth x selection	236.017	1	236.017	6.335	n.s.
growth x lines within[B]	149.017	4	37.254	3.293	*
error[C]	2579.500	228	11.314		

* = P < 0.05 ** = P < 0.01 *** = P < 0.001
Error terms as in Table 6.1

TABLE 6.9 NESTED ANALYSIS OF VARIANCE
Trichome counts - region 3 - males

source	ss	df	ms	F	P
growth temperature	12355.350	1	12355.350	567.410	* * *
selection temperature	1242.150	1	1242.150	71.183	* *
lines within selection[A]	69.800	4	17.450	1.802	n.s.
growth x selection	173.400	1	173.400	7.963	*
growth x lines within[B]	87.100	4	21.775	2.249	n.s.
error[C]	2207.800	228	9.683		

* = P < 0.05 ** = P < 0.01 *** = P < 0.001
Error terms as in Table 6.1

TABLE 6.10 NESTED ANALYSIS OF VARIANCE
Trichome counts - region 3 - females

source	ss	df	ms	F	P
growth temperature	10680.004	1	10680.004	718.177	***
selection temperature	1387.204	1	1387.204	265.291	***
lines within selection[A]	20.917	4	5.229	.656	n.s.
growth x selection	130.538	1	130.538	8.778	*
growth x lines within[B]	59.483	4	14.871	1.864	n.s.
error[C]	1818.850	228	7.977		

* = P < 0.05 ** = P < 0.01 *** = P < 0.001
Error terms as in Table 6.1

TABLE 6.11 NESTED ANALYSIS OF VARIANCE
Total dorsal cell number estimates - region 1 - males
(Data in columns 'ss' and 'ms' are expressed as $x10^7$)

source	ss	df	ms	F	P
growth temperature	3.14028	1	3.14028	1102.39	***
selection temperature	0.0778164	1	0.0778164	1.337	n.s.
lines within selection[A]	0.2328410	4	0.0582102	1.338	n.s.
growth x selection	0.0089706	1	0.0089706	3.149	n.s.
growth x lines within[B]	0.0113947	4	0.0028486	0.065	n.s.
error[C]	9.91614	228	0.0434918		

* = P < 0.05 ** = P < 0.01 *** = P < 0.001
Error terms as in Table 6.3

TABLE 6.12 NESTED ANALYSIS OF VARIANCE
Total dorsal cell number estimates - region 1 - females
(Data in columns 'ss' and 'ms' expressed as $x10^7$)

source	ss	df	ms	F	P
growth temperature	6.07010	1	6.07010	35.244	**
selection temperature	4.97415	1	4.97415	13.967	*
lines within selection[A]	1.42454	4	0.3561341	5.01	***
growth x selection	0.8624145	1	0.8624145	5.007	n.s.
growth x lines within[B]	0.6889258	4	0.1722314	2.423	*
error[C]	16.2070	228	0.0710833		

* = P < 0.05 ** = P < 0.01 *** = P < 0.001
Error terms as in Table 6.1

TABLE 6.13 NESTED ANALYSIS OF VARIANCE
Total dorsal cell number estimates - region 2 - males
(Data in columns 'ss' and 'ms' are expressed as $x10^7$)

source	ss	df	ms	F	P
growth temperature	22.6756	1	22.6756	153.031	***
selection temperature	0.0664864	1	0.0664864	0.717	n.s.
lines within selection[A]	0.3707998	4	0.0926999	1.404	n.s.
growth x selection	0.0040716	1	0.0040716	0.027	n.s.
growth x lines within[B]	0.5927075	4	0.1481768	2.245	n.s.
error[C]	15.0488	228	0.0660032		

* = P < 0.05 ** = P < 0.01 *** = P < 0.001
Error terms as in Table 6.1

TABLE 6.14 NESTED ANALYSIS OF VARIANCE
Total dorsal cell number estimates - region 2 - females
(Data in columns 'ss' and 'ms' expressed as $x10^7$)

source	ss	df	ms	F	P
growth temperature	0.0380727	1	0.0380727	0.2347	n.s
selection temperature	1.75030	1	1.75030	7.0796	n.s.
lines within selection[A]	0.9889233	4	0.2472308	3.0688	*
growth x selection	0.8897735	1	0.8897735	5.4854	n.s.
growth x lines within[B]	0.6488255	4	0.1622063	2.0134	n.s.
error[C]	18.3683	228	0.0805625		

* = P < 0.05 ** = P < 0.01 *** = P < 0.001
Error terms as in Table 6.1

TABLE 6.15 NESTED ANALYSIS OF VARIANCE
Total dorsal cell number estimates - region 3 - males
(Data in columns 'ss' and 'ms' are expressed as $x10^7$)

source	ss	df	ms	F	P
growth temperature	2.00734	1	2.00734	31.984	**
selection temperature	0.1030053	1	0.1030053	3.8701	n.s.
lines within selection[A]	0.1064617	4	0.0266154	0.5517	n.s.
growth x selection	0.0003864	1	0.0003864	0.00616	n.s.
growth x lines within[B]	0.2510424	4	0.0627606	1.3008	n.s.
error[C]	10.9999	228	0.0482453		

* = P < 0.05 ** = P < 0.01 *** = P < 0.001
Error terms as in Table 6.1

TABLE 6.16 NESTED ANALYSIS OF VARIANCE

Total dorsal cell number estimates - region 3 - females (Data in columns 'ss' and 'ms' expressed as $x10^7$)

source	ss	df	ms	F	P
growth temperature	0.1156898	1	0.1156898	0.8659	n.s.
selection temperature	1.32	1	1.32	21.186	*
lines within selection[A]	0.2492165	4	0.0623041	1.1267	n.s.
growth x selection	0.4833965	1	0.4833965	3.6182	n.s.
growth x lines within[B]	0.5343991	4	0.1335997	2.4159	*
error[C]	12.608	228	0.0552981		

* = P < 0.05 ** = P < 0.01 *** = P < 0.001

Error terms as in Table 6.1

Chapter 7 THE EFFECTS OF THERMAL SELECTION ON ADULT LIFE HISTORY CHARACTERS

7.1 Introduction

It has been known for a long time that temperature is a major environmental factor in the determination of longevity in Drosophila. The classical paper by Loeb and Northrop (1917) examined the influence of food and temperature on the duration of life in D. melanogaster, and demonstrated that ageing in D. melanogaster was markedly accelerated by keeping organisms at temperatures in the upper viable range. Between 10-30°C, the rate of larval and pupal development was slower and adult lifespan longer at lower temperatures. From these results Loeb and Northrop (1917) inferred a temperature coefficient (Q_{10}) for the duration of life of between 2 and 3.

These results were confirmed by Pearl (1928) and Alpatov and Pearl (1929), who showed, in addition, that the lifespan of the adult kept at a given temperature was influenced by the temperature at which the larval and pupal development had occurred. An increase in temperature resulted in an increased metabolism and reduced lifespan. Pearl (1928) developed the 'rate of living' theory, which states that the rate of senescence is causally related to metabolic intensity. Pearl (1928) proposed that the decrease of lifespan occuring in older flies was due to the accumulation of some toxin or the destruction of "ageing preventative substances".

Most studies consider that only adults are subject to ageing. However, preimaginal factors are known to exert a strong

and lasting influence on adult life expectancy. For instance, in D. melanogaster it is known that developmental temperature influences life expectancy of the adults, and most studies show decreased adult longevity with increasing preimaginal temperature (e.g. Alpatov and Pearl 1929; Burcombe and Hollingsworth 1970; Lints 1971; Lints and Lints 1971a). A study by Strehler (1961, 1962) showed that larvae reared at 18°C exhibited greater mean adult lifespans than those reared at 25°C, when the adults were kept at 18°, 25° or at 28°C. Muller (1963) reviewed the relationship between preimaginal development and adult lifespan in Drosophila and argued that ageing was a built-in consequence of development and could be directly related to preimaginal conditions. Lints and Lints (1971 b) manipulated preimaginal temperature and crowding and found a strong negative correlation between growth rate and lifespan. They argued that Drosophila adult lifespan could be causally related to duration of development. This seemed to confirm the earlier studies in Drosophila (e.g. Alpatov and Pearl 1929; Burcombe and Hollingsworth 1970).

The problem in this work is that many studies examined only a restricted range of temperatures. A wider range of temperatures was investigated by Cohet (1975) and Cohet and David (1978), who found that the lifespan of unmated female D. melanogaster kept at 25°C increased from 48 days for flies reared at 12°C to 85 days for flies reared at 17°C. At higher developmental temperatures, a decrease in lifespan was observed, so that longevity of flies reared at 32°C was about 50 days. Similar results to those of Cohet (1975) were found for both D. melanogaster and D. simulans (David and Clavel 1967;

McKenzie 1978; Economos and Lints 1984a, 1984b, 1986a, 1986b). In both these species, adult life expectancy was maximal after intermediate developmental temperatures. The results of Cohet (1975) and Economos and Lints (1984a, 1984b, 1986a, 1986b) show that intermediate growth temperatures can be considered as optimal since they produce adults with the greatest life expectancy and lowest rate of ageing.

In an attempt to check the predictions of the rate of living theory, Clarke and Maynard Smith (1961a, b) and Maynard Smith (1963) transferred D. subobscura from one temperature to another. They maintained adults at 30°C for periods of up to half of their life expectancy at 30°C and then transferred them to 20°C. The mean lifespan of these transferred flies was similar to that of flies kept continuously at 20°C. Transferred females showed an even longer lifespan than usual for females kept continously at 20°C. This may have been due to the sterilizing effect of high temperatures (Maynard Smith 1958). In a series of similar experiments D. subobscura were kept at 26°C for about half of their expected lifespan and then transferred to 20°C. Again, lifespan of the transferred flies was similar to that of flies kept constantly at 20°C. If transfer was made in the first half of the expected lifespan, up to 24 days, then the lifespan of transferred flies was similar to flies kept throughout at 20°C. Transfers made at various ages after 24 days produced flies that lived for much shorter periods. The suggestion was that the duration of life in Drosophila was the result of two successive but independent phases of life. Only the latter part of the lifespan was temperature dependent (the dying phase) and the earlier part of life (the ageing phase) was independent of

temperature and metabolic rate. This was termed the 'threshold theory of ageing', which proposes that ageing leads to a decline in the vitalty of an individual which continues until the vitalty falls below a threshold level, after which the individual enters the dying phase. According to the threshold theory of ageing, differences in longevity observed at different temperatures would be due to differences in the level of vitality needed for survival and not simply differences in the rate of ageing.

The results of Clarke and Maynard Smith (1961a, b) have however proved difficult to reproduce, therefore the broader implications of their work have largely been rejected (Sohal 1986). The main postulate of the rate of living theory, that rates of metabolism and ageing are linked together appears to be supported by the majority of recent experimental evidence (reviewed in Sohal 1986). At higher temperatures D. melanogaster has been shown to have increased oxygen utilization and significant accelerated loss of vitality at (Miquel et al. 1976), together with shorter lifespan, ribosomal loss, changes in malphigian tubule structure and lipofuscin-ceroid accumulation.

Most previous studies on adult longevity in Drosophila have only involved manipulation of rearing and environmental temperature. There have been few studies of the evolution of adult longevity using thermally selected lines, or natural populations from different latitudes. The first experiment presented in this chapter tested the effects on adult longevity of prolonged thermal selection at 16.5°C and 25°C, by rearing and maintaining flies from each selection regime at each temperature.

As discussed previously (Chapter 1) female fecundity in Drosophila melanogaster varies both with latitude and altitude in field conditions, decreasing with increasing latitude and altitude (Lemeunier et al. 1986). A large genetic component to this variation in fecundity is revealed by standard rearing experiments under controlled laboratory conditions (e.g. David and Capy 1988; Capy et al. 1983; 1986).

Latitudinal variations in reproductive capacity are also reflected in other morphological and physiological traits such as ovariole number (David 1970; David and Bocquet 1975b), which generally increases with latitude in both D. melanogaster and D. simulans (Capy et al 1983; David and Bocquet 1975a; 1975b), when examined under controlled laboratory conditions at 25°C. These clines are repeated on different continents (David and Capy 1988). It is possible that these clines could be rapidly established, as David and Bocquet (1975b) indicate that less than 25 generations of artificial directed selection on equatorial strains are needed to reach the higher ovariole numbers of temperate strains.

In addition to differences in female fecundity between strains from different latitudes, numerous laboratory studies have demonstrated that in D. melanogaster developmental temperature influences egg production (David et al. 1975; Cohet and David 1978; Fogleman 1979; Giesel et al. 1982). At high temperatures (> 31°C), the fecundity of D. melanogaster females and the percentage of pupae eclosing is close to zero.

The general assumption in the theory of evolution of life histories is one of trade offs between various life history components (Stearns 1976; Charlesworth 1980). For instance, a

trade-off may occur between present and future reproduction, e.g. early reproduction may carry the cost of a reduction in future reproductive capacity. These reproductive costs may be revealed in terms of inverse relationships with other life history components such as adult survival.

The present work examined the egg production of females from both thermal selection regimes, reared at both temperatures and continually exposed to males throughout their lives. In addition I calculated the total lifetime egg production for each individual female. This is of obvious evolutionary importance as a major component of the overall fitness of a female, determining the extent to which her genotype contributes to future generations. I have also examined directly the longevity of females used in that experiment, to determine any relationship between egg production and longevity in females from the two selection regimes. I also examined the percentage of eggs laid by each individual female that gave rise to adult progeny, and expressed this as egg-hatchability. The measures of adult longevity and of female fecundity and fertility should shed light on any divergence or thermal adaptation between flies from the two selection regimes, in respect of female fecundity.

7.2 Materials and Methods

(a) Fly stocks and culture.

All three replicate lines from both the 16.5°C and 25°C selection regimes were examined at both environmental temperatures. The adults used in this experiment were obtained from vials containing 100 larvae each, which were used to determine development time (see chapter 3.3.1 for details of materials and methods). The experimental flies had therefore experienced one generation of controlled density rearing, and two generations of controlled temperature rearing. The flies and their progeny were maintained at the appropriate experimental temperature.

The adults which emerged from the vials were collected as virgins after eclosion, over a period of two days. Sexes were stored separately in fresh vials containing Lewis medium for 24 hours from their time of eclosion.

(b) Measurement of adult longevities.

Adults from each replicate development vial were sorted under carbon dioxide anaesthesia, into each of four lightly yeasted vials of Lewis medium at a density of 5 males and 5 females per vial, giving a total of 12 vials for each replicate selection line at each experimental temperature. All experimental vials were examined every day for deaths, and dead flies were removed by aspiration. As deaths occurred throughout the experiment the surviving adults were kept at a density as close to 10 per vial as possible at the original sex

ratio. At both 16.5°C and 25°C the flies were transferred to fresh lightly yeasted vials of Lewis medium every two days.

(c) Measurement of fecundity, fertility and survival of females

The experimental females were collected from the development time vials (see above) and sorted into each of fifteen vials containing Lewis medium, with one female and two males per vial. Males used in the experiment were obtained from the 25°C Brighton stock, in the standard way described previously (Section 3.3). The purpose of having two males with each female was to guard against the possibility of male infertility, which would interfere with subsequent progeny production. An additional fifteen vials containing one female and two males were also set up as "back-ups" to replace males that died through the course of the experiment. Flies in both groups, experimental and back-up, were transferred to fresh, lightly yeasted vials of Lewis medium every two days. If, at any time during the 2-day egg-laying period, the experimental female died, then the egg count was taken from that vial, but not included in the calculation of mean two or four day egg counts. The egg count was included in the calculation for each individual female's lifetime reproductive success. If an experimental male died during the experiment he was replaced during the transfer to fresh vials by a male from a corresponding back-up vial. After each experimental vial was cleared of flies, the surface of the food medium was examined and the eggs counted using a hand-held tally counter. After all egg counts were completed, the vials

were returned to the controlled temperature rooms until the adult progeny had emerged. The vials were cleared of adults on several occasions over a 2 to 3 day period at 25°C, or over a 5 to 6 day period at 16.5°C, until eclosion was completed in each vial. Progeny were counted and sexed and then discarded.

Hatchability is a complex expression, because it depends on:

(a) The percentage of eggs that are fertilized

and

(b) The percentage of fertilized eggs that survive embryonic, larval and pupal stages.

The percentage of eggs fertilised depends on male and female traits such as courtship ability, sperm quality, willingness to mate and sperm storing ability. This measure assumes that mortality between egg and adulthood did not differ between the selection lines. From observations of survival rates and larval competition in Chapters 3 and 5, at the densities and temperatures involved here, this is a reasonable assumption. Assuming small progeny mortality rates, I took the number of adult progeny produced as a measure of the number of fertile eggs laid.

7.3 Results

(a) Adult longevities

The cumulative probability of survival (the probability of surviving to a given time interval, taking into account withdrawals from the experiment caused by escapes and accidents) for males kept at 25°C are given in Figure 7.1, and for

females in Figure 7.2. The corresponding data for flies kept at 16.5°C are given in Figures 7.3 and 7.4.

The data were analysed using the BMDP 1L statistical package and the test statistic used was the Mantel-Cox. The median survival times together with the first and third quartiles for each replicate line at 25°C and 16.5°C are given in Table 7.1a and 7.1b. The replicate lines from each selection regime were also examined for heterogeneity. The Mantel-Cox statistic and associated P value for the comparisons of replicate cage lines within each selection regime are also given in Tables 7.1a and 7.1b. There were no significant differences in longevity between the replicate cage populations of either selection regime, at either experimental temperature.

In order to determine the significance of any differences in longevity between the selection regimes, three independent comparisons were made between the cage lines from each selection regime using BMDP 1L. For each sex at each experimental temperature, the 25°C and 16.5°C replicate lines were paired for comparison using the replicate numbers allocated before the experiment started to determine the pattern of pairing. The probabilities from these independent pairwise comparisons were then combined (Sokal & Rohlf 1981; pp 779-782). The results of these analyses are given in Tables 7.2 and 7.3 for the experiments at 25°C and 16.5°C respectively. Males and females were analysed separately. Tables 7.2 and 7.3 show that there were significant differences in longevity between females of 16.5°C and 25°C selected lines at both experimental temperatures. At 16.5°C females from the 16.5°C selected replicate lines lived longer than females from the 25°C selected

lines. The situation was reversed at 25°C, with 25°C selected females living longer than the 16.5°C selected females. There was also a significant difference in longevity at 16.5°C between males of the two selection regimes, with 16.5°C selected males living longer than 25°C selected males. However, there was no significant difference in longevity between males of the two selection regimes at 25°C.

(b) Fecundity, fertility and survival of females

The mean and 95% confidence intervals for each two day egg count for females from both selection regimes at 25°C are given in Figure 7.5. Figure 7.6 shows the mean number of eggs laid every four days and 95% confidence intervals at 16.5°C. For each two and four day count a separate non parametric test, a Kruskal Wallis one way analysis of variance, was performed. This analysis included multiple comparisons between all the groups, as defined by Siegel (1988). The results of the analysis are summarised in Tables 7.4 and 7.5. The analysis showed that, at 25°C, females from the 25°C selection regime laid a significantly greater number of eggs per two days for most of their lives, than did females from the 16.5°C selection regime. At the lower experimental temperature, females of the 16.5°C selection regime laid more eggs every four days for most of their lifetime than did females of the 25°C selection regime. In both cases the highly significant differences in egg counts broke down only during the final few days of the experiment, just before the few remaining females died.

The total number of eggs laid by each female was recorded giving a score of lifetime egg-production for that individual. Figure 7.7 gives the mean total number of eggs laid and 95% confidence intervals for each replicate line of both selection regimes at 25°C. The corresponding data for the females at 16.5°C are presented in Figure 7.8. The data were analysed using a 2-way analysis of variance, with selection temperature cross-classified with experimental temperature as fixed main effects, and replicate lines nested within each selection regime. Table 7.6 gives the results of this analysis. There was a significant main effect of experimental temperature. Females from the 16.5°C selection regime had much greater lifetime fecundity at 16.5°C, while females from the 25°C selection regime showed a much smaller change in the opposite direction. There was also a highly significant interaction between experimental and selection temperatures. Females from each selection line had greater fecundity when they were tested at the temperature at which they had been selected There were no significant differences between replicate lines within selection regimes at either experimental temperature.

The number of progeny produced from each vial was recorded. Given that the number of eggs in each vial was known, an estimate of egg hatchability was obtained (this assumes that mortality in the larval and pupal life stages and non-hatching due to infertility did not differ significantly for flies from the different selection regimes, at the densities involved). The analysis of egg hatchability was the same as that used for analysis of egg counts. There were no significant differences in egg hatchability between the two selection regimes at either

experimental temperature. This can be seen clearly by inspection of Figure 7.9 and Figure 7.10.

The number of progeny produced by each female throughout the experiment were totalled and analysed in the same way as for lifetime egg-production. Results for lifetime progeny-production were similar to those for lifetime egg-production, and are given in Table 7.7. There was a significant main effect of experimental temperature, due mainly to the large increase in lifetime progeny production of the 16.5°C selected lines at 16.5°C experimental temperature. Lifetime progeny production of the 25°C selected lines decreased slightly at the lower experimental temperature. There was a significant interaction between replicate lines and experimental temperature. That is, replicate lines within the selection regimes showed significant variation in their response to the two experimental temperatures. There was no significant variation between replicate lines within each selection regime.

The date of death of each female in the fecundity/fertility experiment was recorded and these data were analysed using the BMDP 1L statistics package. The median survival time and first and third quartile for each replicate line at 25°C and 16.5°C are given in Tables 7.8 and 7.9, together with the Mantel Cox statistic and associated P value. These tables show the results of comparison between replicate lines within each selection regime. There was no significant heterogeneity in longevity between the replicate lines. To analyse longevity between selection regimes the 25°C and 16.5°C replicate lines at each experimental temperature were paired for comparison, using numbers allocated before the start of the experiment to determine the

pattern of pairing. The probabilities from these independent pairwise comparisons were then combined using the method outlined by Sokal & Rohlf (1981; pp 779-782). The results of this analysis are given in Table 7.10; this shows that there were no significant differences in longevity between females from the two selection regimes at either experimental temperature. The cumulative probabilities of survival for females at 25°C are given in Figure 7.11 and for females at 16.5°C in Figure 7.12. Both these Figures illustrate clearly that there were no significant differences between the selection regimes in this experiment, in contrast to the results of the first (longevity) experiment (see below).

7.4 Discussion

The well documented effect of experimental temperature on Drosophila adult longevity (see section 7.1), was very obvious in the present study. The longevity of flies from both selection regimes was greater when those flies were reared and maintained at the lower temperature (16.5°C).

The evolutionary response of longevity to temperature was also very marked. At the lower experimental temperature, flies from the 16.5°C selected lines showed greater longevity compared to flies from the 25°C selected lines (Figures 7.3 and 7.4). At 25°C experimental temperature, females from the 25°C selected lines survived significantly longer than did females from the 16.5°C selected lines (Figure 7.2), while there was similar but non-significant effect for males (Figure 7.1). The

effect of selection temperature on longevity was therefore depedent upon the experimental temperature.

The previously observed differences in development time, to eclosion, between the selected lines (section 3.2) are interesting, in relation to the adult longevity data. The faster developing lines (16.5°C selected lines reared at 16.5°C, and 2 5°C selected lines reared at 25°C) also had the greater adult longevity (except for males reared at 25°C, where differences were non-significant). Similarly, these lines also showed the faster larval growth rates (Chapter 4). The longevity of flies from replicate lines of the same selection regime showed no significant differences, in sharp contrast to development times and larval growth measurements, which showed significant heterogeneity between replicate lines within selection regimes (Chapters 3 and 5).

As discussed in Chapter 6, adult body size has been shown to be related to longevity, with larger individuals living longer. As observed earlier, 16.5°C selected flies were larger than 25°C selected flies when reared at either 16.5°C or 25°C. The longevity of 16.5°C selected flies was greater than 25°C selected flies, when reared and maintained at 16.5°C. However, this was not the case at 25°C, where females selected at 25°C showed greater longevity. The relationship between body size and longevity clearly does not hold in this case. It must be noted that previous studies of body size and longevity have usually been conducted at a single temperature, using size selected lines, and do not concern adaptation to specific thermal environments.

The results from the present experiment show that the effect of selection temperature on adult longevity depends upon

the temperature at which longevity is measured. Flies from each selection regime showed signs of adaptation, having evolved characteristics to enable them to survive for longer at the temperature at which they were selected.

There are only a handful of studies similar to the experiments reported here. Mourad (1965) examined longevity among four of six experimental populations of D. pseudoobscura which had been maintained for four and a half years in three different environments (16°C, 25°C, 27°C) for evidence of genetic diversity. There was no evidence for an effect of evolutionary temperature at any of the testing temperatures. (See also David 1988, for a general review of temperature and longevity). There have been no other comparable studies of the effects of laboratory thermal selection on longevity which have shown evidence of adaptation.

Environmental temperature had a considerable effect on female reproduction. At 25°C, egg-production at each sampling interval was much higher early in the life of the females and declined rapidly as the experiment progressed, compared to the experiment conducted at 16.5°C (see Figures 7.5 and 7.6).

Thermal selection had a significant effect upon egg-laying ability and, once again, this life history trait showed clear evidence of adaptation. As with the results for adult longevities, the effects of selection temperature on female fecundity were dependent upon the temperature at which this was tested. The egg-laying ability and lifetime egg-production of 16.5°C selected females was greater than that of 25°C selected females, when tested at 16.5°C (Figures 7.6 and 7.8). The reverse was true when females were reared and maintained at 25°C (Figures 7.5

and 7.7). This is clear evidence of adaptation to temperature, with females from each selection regime showing greater fecundity at each sampling interval, when reared and tested at the temperature at which they had been selected.

Several studies have demonstrated that fecundity is related to other aspects of life history, namely body size (Chapter 6) and adult longevity (Chapter 7.1). For example, Tantaway and Vetukhiv (1960) and Tantaway (1961a) showed that, in D. pseudoobscura, larger females lived longer and laid more eggs than smaller females. Similarly, Partridge and Fowler (1992) found that D. melanogaster selected for greater longevity were larger at eclosion and laid more eggs during their lifetime. However, in D. melanogaster selected to be larger at eclosion, longevity was reduced with more eggs being laid early on in life (Hillesheim and Stearns 1992). It is important here to distinguish the character being selected. Hillesheim and Stearns, in selecting for heavier flies at eclosion, were producing flies with larger ovarioles. These flies laid more eggs early on in their life, at the expense of longevity, and therefore died younger. Partridge and Fowler (1992) were selecting for flies that lived longer. These flies did not lay more eggs early in life, but it is likely that they put more resources into other aspects of physiology such as storage and somatic maintenance. Hillesheim and Stearns (1992) found that larger flies laid more eggs early in life, and had lower lifespan compared to smaller flies, which lived longer and laid more eggs later in life. It is likely that female body size is an important component of fitness, because it shows a positive genetic and phenotypic correlation with fecundity and longevity (Robertson 1957; Tantaway and El-Helw

1966; Tantaway and Rakha 1964; Partridge and Farquhar 1983; Heed and Mangan 1986).

In the present study, 16.5°C selected females were bigger but less fecund than 25°C selected females, when both were reared and maintained at 25°C. Given that large size is positively correlated with increased longevity and fecundity in Drosophila (Robertson 1957; Tantaway & Vetukhiv 1960; Partridge & Farquhar 1983), one might expect larger flies in the experiments reported here to be longer lived and have greater fertility. Flies from the 16.5°C selected lines are larger than 25°C selected lines, when reared at either 16.5°C or 25°C. However, the correlation between body size and fitnes changes direction between the two growth temperatures. This could mean that thermal selection on body size involves different genes than thermal selection on other fitness components, and therefore size differences between the selection regimes don't play any part in determining differences in longevity and fecundity between selection regimes.

The adult progeny counts for each vial were combined with egg counts to give a measure of the percentage of eggs hatching. Subsequent analysis showed no significant differences in egg hatchability between the selection regimes at either experimental temperature.

In contrast to the results for adult female longevity given in section 7.3(a), in the fecundity/fertility experiment there were no significant differences in longevity between the females from the two selection regimes, at either environmental temperature. These two contrasting results cannot be due to differences in pre-adult rearing conditions, as both sets of

females came from the same source. Any 'unknown factor' or adverse environmental condition (possibly variations in experimental temperature) would have had to act differentially, reducing or increasing the longevity of one group in relation to the other. Several factors may have contributed to this anomalous result. The sample size in the previous longevity experiment was much larger than that used here (60 females for each replicate line in the former case compared with only 15 females per replicate line in the present experiment). Such low sample sizes make the detection of any differences in longevity between the selected lines that much more difficult. There were also differences between the longevity experiment and the fertility/fecundity experiment in the total number of adults in each vial and the male:female ratios (5 males : 5 females compared with 2 males : 1 female), which could possibly have contributed to the negative result in the latter experiment.

It is well known that longevity in Drosophila is reduced by sexual activity (mating, copulation, egg production). Virgin females and males live longer than mated flies (e.g. Cohet and David 1976; Partridge and Farquhar 1981; Partridge and Andrews 1985; Partridge et al. 1986). For example, Biliewicz (1953) found that in D. melanogaster, virgin females lived for approximately twice as long as mated females, but laid about the same number of eggs per lifetime (see also Partridge et al. 1986).

Egg production is costly to female Drosophila, mainly by reducing their lifespan. For example, Maynard Smith (1958) found that virgin females (and ovaryless mutants) of D. subobscura lived for significantly longer than normal mated

females at 20°C. Life was prolonged when females were exposed to 30.5°C for a short time, possibly due to the reduction in the rate at which such exposed females subsequently lay eggs. The exposure of the young females to high temperature caused a partial regression of the ovaries and a permanent change in subsequent behaviour and rate of egg laying. Similarly, sterilization by X-irradiation has been found to extend life expectancy in D. subobscura (Lamb 1964). Using either high temperature or X-irradiation to sterilize females did not prolong life in mutant ovaryless females (Maynard Smith 1958; Lamb 1964). This is a strong case for egg production itself having a significant effect on lifespan.

By determining genetic correlations between various life history variables, Rose and Charlesworth (1980, 1981) found a negative correlation between fertility and longevity, as did Rose (1984) and Luckinbill et al. (1984). Using a different experimental approach (phenotypic manipulation), Partridge et al. (1987c) also found a negative correlation between early fertility and longevity. Most studies involve manipulation of egg production, with the cost of egg production identified in terms of reduced lifespan.

Costs of reproduction have also been examined by comparing the survival of females whose exposure to males was varied. These types of studies have revealed that virgin females lived longer than inseminated individuals (see Partridge 1986). However, although the inseminated females lay more eggs than the unmated females early in life, because the virgin females live longer, their total lifetime egg production can match or even exceed that of inseminated females (Bilewicz 1953; David 1963;

Bouletreau 1978; Partridge et al. 1986). The implication is that the observed differences in mortality between these two groups of females cannot be explained simply on the basis of egg production alone. However, some qualitative differences between the eggs produced by virgins and by mated females, could make the former less costly to produce (David 1963).

Although there are direct costs of egg production, exposure to males may itself directly affect female longevity (Partridge et al. 1987c). Female D. melanogaster exposed to males intermittently had greater lifetime progeny totals and greater lifespans than females exposed to males continously. These difference cannot be due to a higher production of unfertilised eggs by the intermittently exposed females, because egg hatchability results from the two groups are similar. Hence continous exposure to males, in itself, reduces the longevity and lifetime progeny production of females: there must be some inherent cost to mating (Partridge et al. 1987c; Fowler and Partridge 1989).

Female Drosophila that produce eggs at a high rate tend to remate more frequently (Gromko and Gerhart 1984). Similarly, Bellen and Kiger (1987) found that in D. melanogaster, dunce mutant females had a much higher remating frequency than the wild-type females, and had a much greater reduction in lifespan when exposed to males, than is the case in wild-type females. Since female D. melanogaster are known to remate frequently in both the laboratory and in the wild (Maynard Smith 1958; Newport and Gromko 1984; Griffiths et al. 1983; Marks et al. 1988), this cost of mating may be a significant factor which influences female longevity.

The life history traits examined in this chapter exhibit a considerable phenotypic plasticity in response to temperature. It is possible that there may be a trade off between the two characters at the two temperatures. Adaptation to one temperature may involve loss of adaptation to the other, due to narrow thermal limits of enzymes. Alternatively, mutation pressure may lower adaptation to temperatures which the fly no longer encounters, as a result of being confined to one particular thermal regime.

One might expect that the well documented life-shortening effects of higher environmental temperatures would lead to the evolution of damage avoidance/repair mechanisms in the high temperature selected lines. For instance, it is known that higher metabolic rates cause an increase in the rate of oxygen reduction and in the generation of free radicals and hyperoxides (Chance et. al. 1979; Halliwell 1984), which are known to cause damage to cellular structures (Brawn and Fridovitch 1980). Several authors have suggested that the deleterious effects of high temperatures and metabolic rates may be due to radical-induced unrepaired cellular damage (Harman 1972; Miquel et al. 1980; Fleming et al. 1982; Miquel et al. 1982). Recent work indicates that metabolic potential may be related to the efficiency of antioxidants and reparative mechanisms. Organisms with high levels of antioxidants in relation to their metabolic potential, and those organisms which have an ability to quickly repair damage due to oxidants generally live longer (Cutler 1984; Fleming 1986; Sohal 1984a, 1984b, 1985a, 1985b).

Possession of effective reparative mechanisms would be reflected in the higher survival of high temperature lines, at

both high and low environmental temperatures. This did not occur in the present thermal selection experiment. Likewise, if evolution at 16.5°C had lead to a greater allocation of resources to fertility, this would have been apparent at both experimental temperatures. This did not occur, but as previously mentioned this may simply reflect mutation pressure leading to loss of adaptation to the temperature not encountered, or different thermal for enzymes involved in damage limitation and/or repair. Thus,in the latter case even if 25°C selected flies were allocating more resources to repair, this might not be reflected in higher survival at 16.5°C.

Table 7.1. BMDP analysis of survival of replicate lines at (a) 25°C and (b) 16.5°C. (Median survival time in days)

(a)

selection regime (MALES)	cage line	median survival time	n	1st Quartile	3rd quartile	Mantel-Cox Statistic	D.F.	P
16.5°C	1	31	60	21	42	0.790	2	0.6736
	2	35	55	18	44			
	3	29	59	18	43			
25°C	1	35	51	24	43	2.041	2	0.3604
	2	30	53	20	39			
	3	33	60	26	38			
(FEMALES) 16.5°C	1	30	57	19	39	1.522	2	0.4673
	2	33	54	22	41			
	3	27	58	19	38			
25°C	1	36	54	24	44	5.265	2	0.0719
	2	39	55	24	47			
	3	33	54	24	42			

(b)

selection regime (MALES)	cage line	median survival time	n	1st Quartile	3rd quartile	Mantel-Cox Statistic	D.F.	P
16.5°C	1	75	60	48	87	5.114	2	0.0776
	2	73	55	54	84			
	3	64	59	42	82			
25°C	1	59	51	45	83	0.261	2	0.8778
	2	66	53	43	83			
	3	61	60	41	80			
(FEMALES) 16.5°C	1	73	57	44	85	3.507	2	0.1732
	2	74	54	50	83			
	3	76	58	48	95			
25°C	1	59	54	29	80	0.142	2	0.9317
	2	63	55	37	78			
	3	65	54	38	78			

BMDP used only the number of deaths in the calculation of means and medians, and ignored withdrawals. This accounts for the variation from n=60 in column 4 of the table.

Table 7.2 BMDP analysis of longevity between the selection regimes at 25°C.

	comparison	Mantel-Cox	P	ln P	Total	X^2	P
Males	25.1 - 16.1	0.903	0.3419	-1.0732			
	25.2 - 16.2	0.843	0.3585	-1.0258	-2.9732	5.9464	N.S.
	25.3 - 16.3	0.658	0.4172	-0.8742			
Females	25.1 - 16.1	7.879	0.0050	-5.2983			
	25.2 - 16.2	8.808	0.0030	-5.8091	-14.3749	28.7498	< 0.001
	25.3 - 16.3	4.300	0.0381	-3.2675			

The calculation is based on the fact that -2 ln P is distributed as X^2.
X^2 is calculated as -2Σ ln P
P is calculated with 2k degrees of freedom. (Where k=the number of independent tests)

Table 7.3 BMDP analysis of longevity between the selection regimes at 16.5°C.

	comparison	Mantel-Cox	P	ln P	Total	X^2	P
Males	25.1 - 16.1	6.540	0.0105	-4.5564			
	25.2 - 16.2	1.950	0.1626	-1.8165	-6.9414	13.8828	< 0.05
	25.3 - 16.3	0.329	0.5664	-0.5685			
Females	25.1 - 16.1	6.376	0.0116	-4.4567			
	25.2 - 16.2	9.403	0.0022	-6.1193	-17.9946	35.9892	< 0.001
	25.3 - 16.3	11.893	0.0006	-7.4186			

Calculations as in Table 7.2

Table 7.4 Kruskal Wallis one-way analysis of variance (Egg counts from each sampling interval at 25°C)

Day	Kruskal Wallis test statistic	D.F.	P
2	50	5	****
4	42.54	5	****
6	29.81	5	****
8	56.44	5	****
10	62.71	5	****
12	53.71	5	****
14	58.84	5	****
16	65.60	5	****
18	64.61	5	****
20	64.82	5	****
22	60.37	5	****
24	57.08	5	****
26	47.04	5	****
28	40.96	5	****
30	38.34	5	****
32	38.79	5	****
34	23.88	5	***
36	20.80	5	***
38	7.84	5	NS
40	8.69	5	NS
42	1.71	5	NS

Table 7.5 Kruskal Wallis one-way analysis of variance (Egg counts from each sampling interval at 16.5°C)

Day	Kruskal Wallis test statistic	D.F.	P
4	62.08	5	****
8	52.73	5	****
12	65.94	5	****
16	49.98	5	****
20	53.98	5	****
24	35.83	5	****
28	44.88	5	****
32	33.05	5	****
36	45.66	5	****
40	58.04	5	****
44	55.22	5	****
48	44.61	5	****
52	49.49	5	****
56	38.39	5	****
60	38.45	5	****
64	29.95	5	****
68	24.79	5	***
72	21.22	5	***
76	11.61	5	*
80	10.85	5	NS
84	5.52	5	NS

Table 7.6 Two-way analysis of variance on lifetime egg-production of females from both selection regimes at both experimental temperatures.

source	ss	df	ms	F	P
experimental temperature	2804260	1	2804260	13.437	*
selection temperature	88489	1	88489	0.688	n.s.
lines within selection[A]	514281	4	128570	1.346	n.s.
experimental x selection	7481500	1	7481500	35.849	**
experimental x lines within[B]	834789	4	208697	2.184	n.s.
error[C]	16051900	168	95547		

* = P < 0.05 ** = P < 0.01 *** = P < 0.001

A .....Lines within selection is the correct error term for selection temperature.

B.....Experimental x lines within selection is the correct error term for both experimental temperature and for experimental x selection.

C.....Error is the correct error term for lines within selection and experimental x lines within.

Table 7.7 Two-way analysis of variance on lifetime progeny-production of females from both selection regimes at both experimental temperatures.

source	ss	df	ms	F	P
experimental temperature	2401710	1	2401710	20.308	*
selection temperature	88445	1	88445	1.055	n.s.
lines within selection[A]	335415	4	83854	1.939	n.s.
experimental x selection	3415790	1	3415790	28.882	**
experimental x lines within[B]	473064	4	118266	2.735	*
error[C]	7265850	168	43249		

* = P < 0.05 ** = P < 0.01 *** = P < 0.001

Error terms as in Table 7.6.

Table 7.8 BMDP analysis of female survival of replicate lines at 25°C - fecundity/fertility experiments. (Median survival time in days)

selection regime	cage line	median survival time	n	1st Quartile	3rd Quartile	Mantel-Cox Statistic	D.F.	P
16.5°C	1	34.00	15	24.00	38.00	1.254	2	0.571
	2	36.00	14	26.00	40.00			
	3	36.00	14	24.00	40.00			
25°C	1	38.00	15	30.00	42.00	4.011	2	0.103
	2	36.00	14	28.00	40.00			
	3	34.00	15	30.00	40.00			

Table 7.9 BMDP analysis of female survival of replicate lines at 16.5°C - fecundity/fertility experiments. (Median survival time in days)

selection regime	cage line	median survival time	n	1st Quartile	3rd Quartile	Mantel-Cox Statistic	D.F.	P
16.5°C	1	68.00	13	52.00	84.00	3.126	2	0.275
	2	68.00	14	52.00	80.00			
	3	60.00	14	44.00	80.00			
25°C	1	60.00	13	44.00	76.00	2.641	2	0.300
	2	60.00	13	48.00	80.00			
	3	64.00	12	48.00	76.00			

Table 7.10 BMDP analysis of female longevity between the selection regimes - fecundity/fertility experiments.

Temperature	comparison	Mantel-Cox	P	ln P	Total	X^2	P
25°C	25.1 - 16.1	2.674	0.1037	-2.2662			
	25.2 - 16.2	0.165	0.6850	-0.3783	-2.8925	5.785	NS
	25.3 - 16.3	0.078	0.7803	-0.2480			
16.5°C	25.1 - 16.1	1.098	0.2948	-1.2214			
	25.2 - 16.2	0.038	0.8454	-0.1679	-2.0921	4.1842	NS
	25.3 - 16.3	0.465	0.4952	-0.7028			

The calculation is based on the fact that -2 ln P is distributed as X^2.

X^2 is calculated as $-2\Sigma \ln P$

P is calculated with 2k degrees of freedom. (Where k=the number of independent tests)

Figure 7.1 Cumulative survival probabilities for males at 25°C.

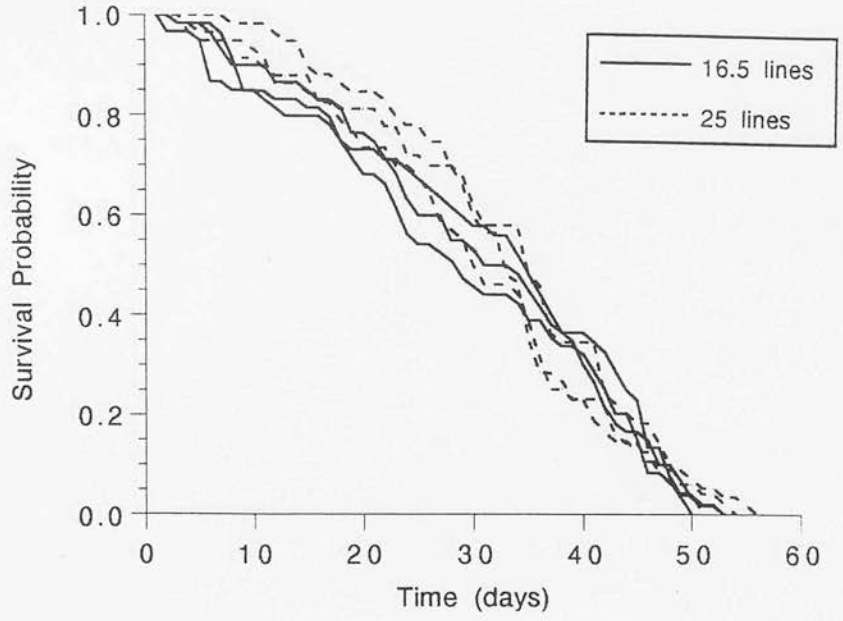


Figure 7.2 Cumulative survival probabilities for females at 25°C.

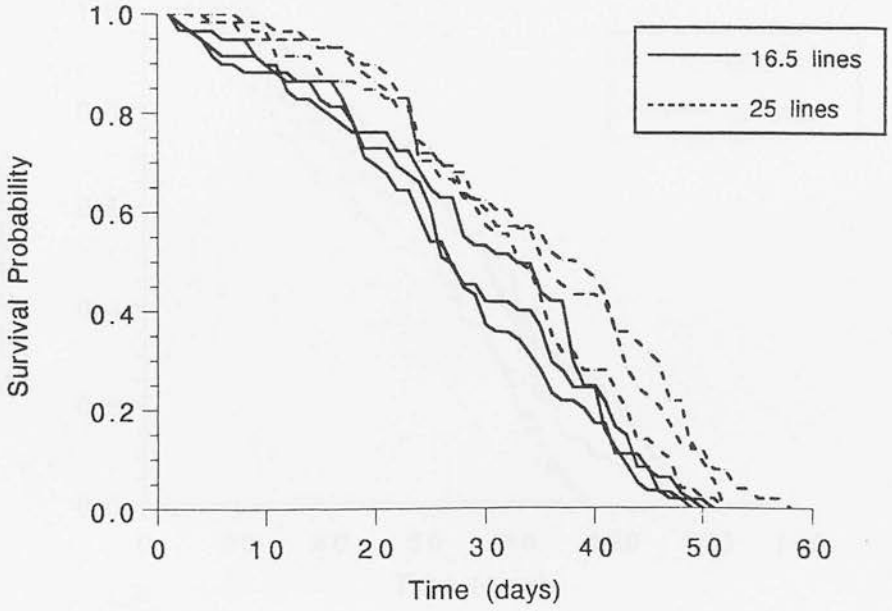


Figure 7.3 Cumulative survival probabilities for males at 16.5°C.

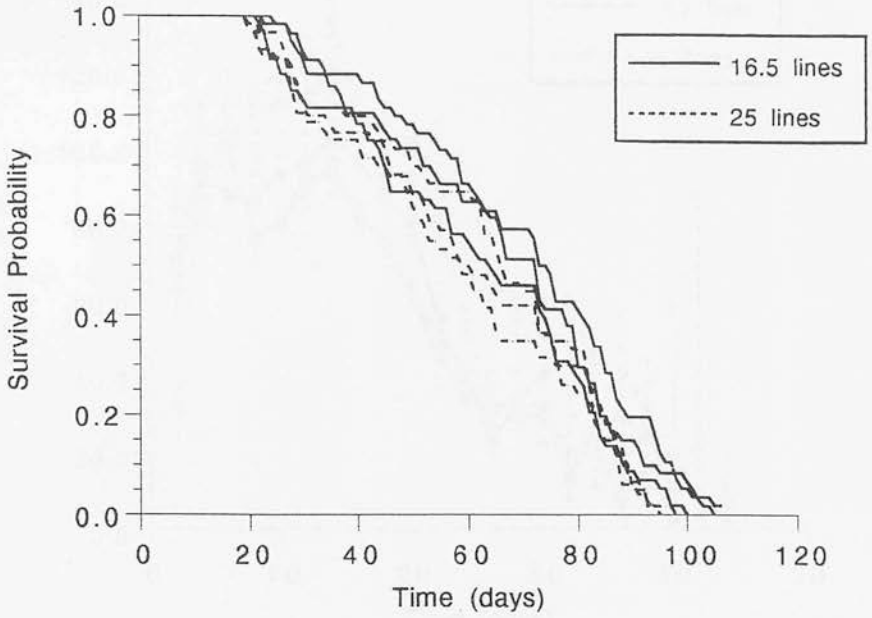


Figure 7.4 Cumulative survival probabilities for females at 16.5°C.

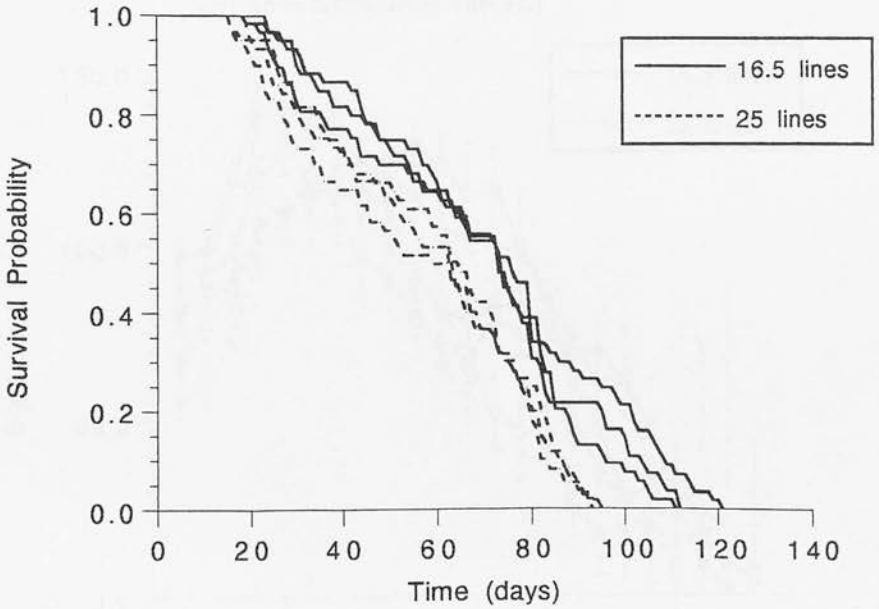


Figure 7.5 Mean two day eggcount at 25°C.
(+/- 95% confidence interval)

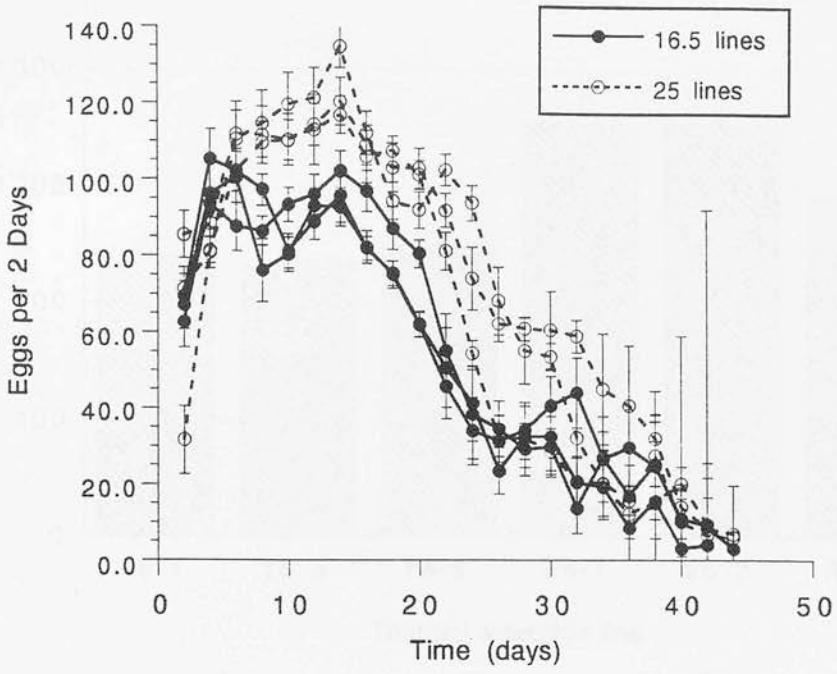


Figure 7.6 Mean four day eggcount at 16.5°C.
(+/- 95% confidence interval)

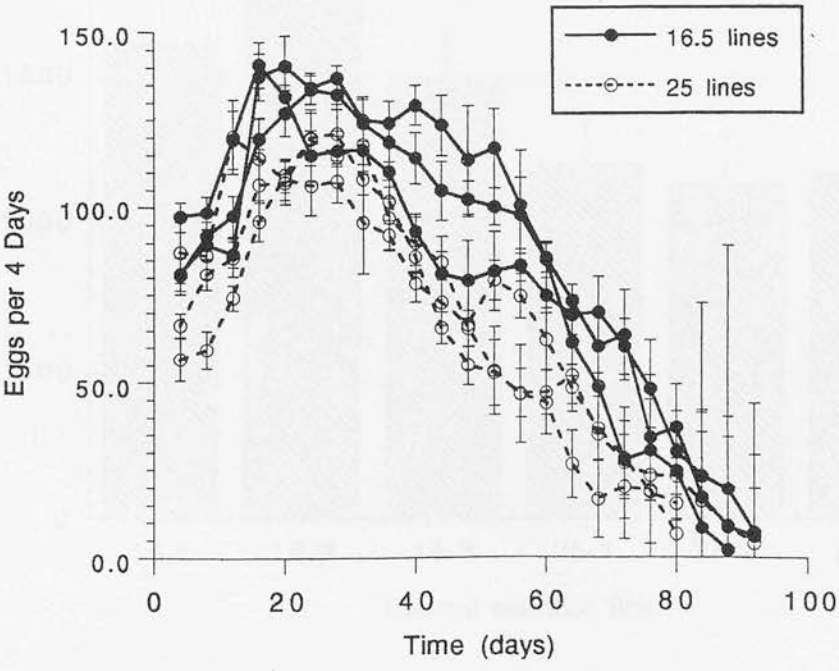


Figure 7.7 Mean lifetime egg-production by females at 25°C.
(+/- 95% confidence limits)

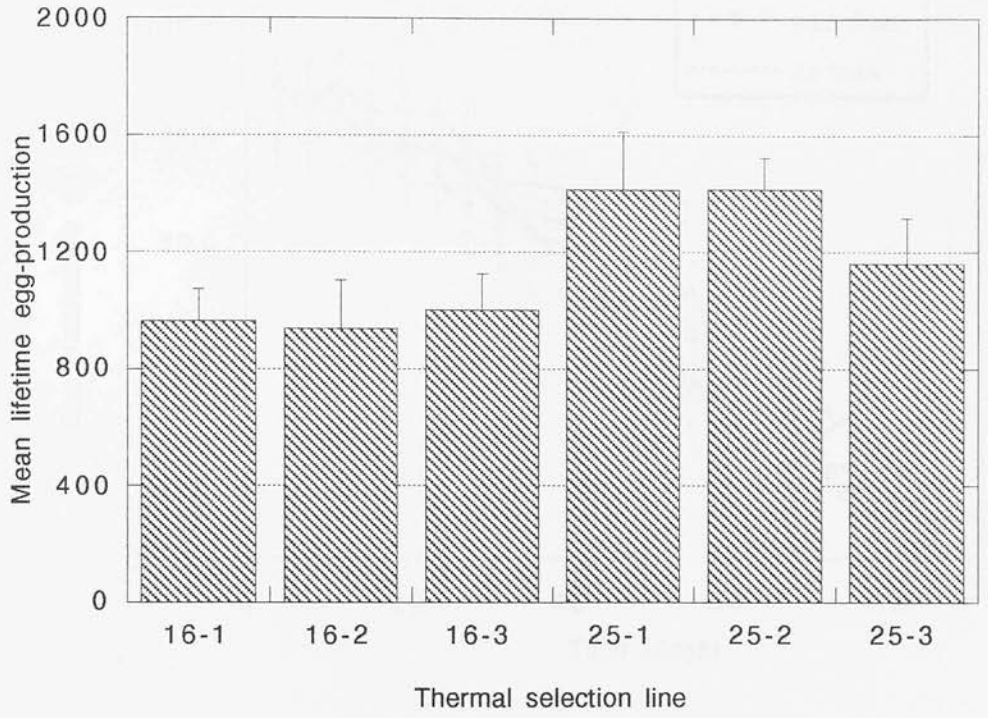


Figure 7.8 Mean lifetime egg-production by females at 16.5°C.
(+/- 95% confidence limits)

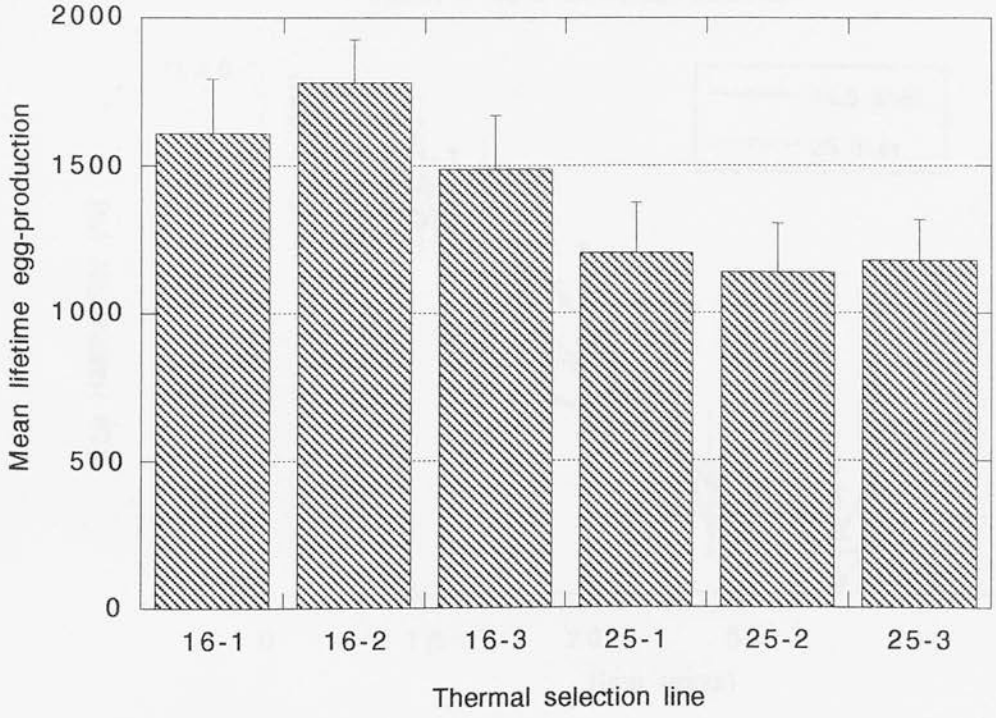


Figure 7.9 Egg hatchability at 16.5°C.
(mean +/- 95% confidence interval)

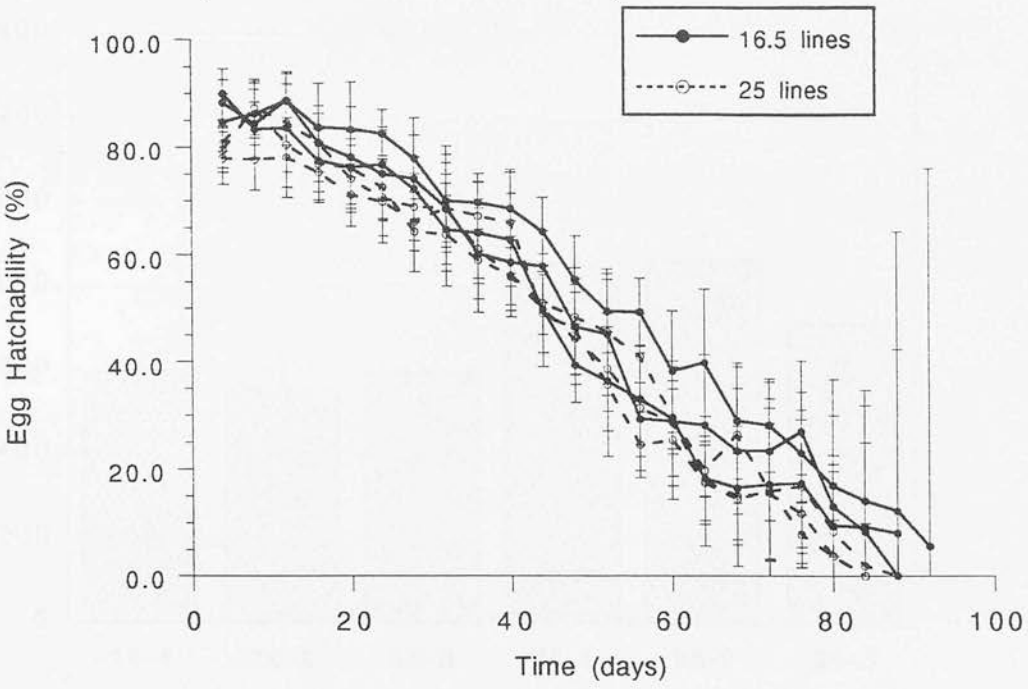


Figure 7.10 Egg hatchability at 25°C.
(mean +/- 95% confidence interval)

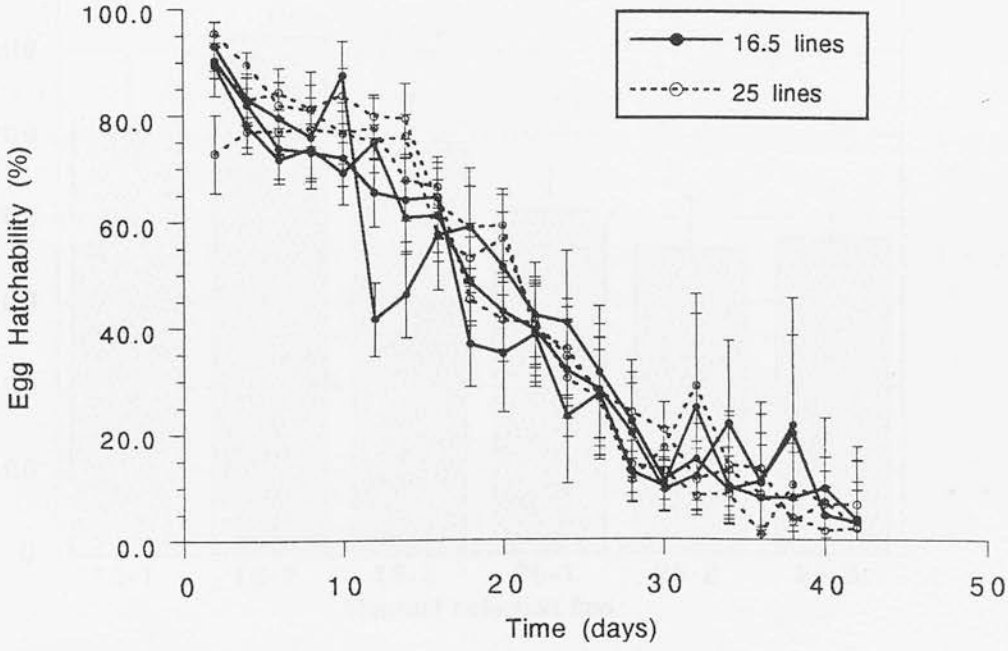


Figure 7.11 Mean lifetime progeny-production by females at 25°C. (+/- 95% confidence limits)

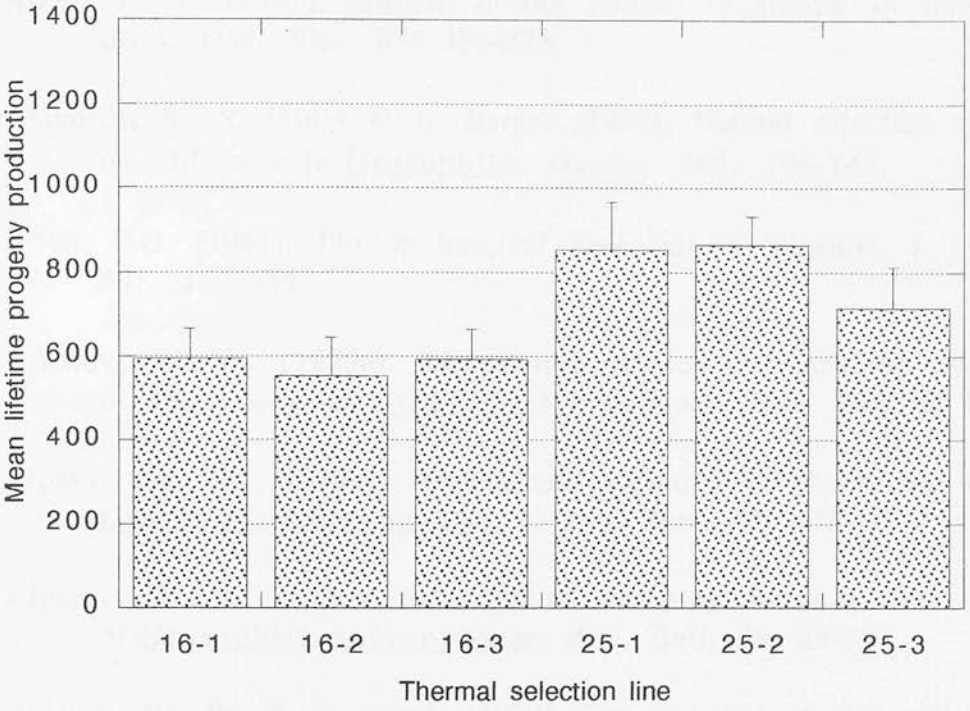
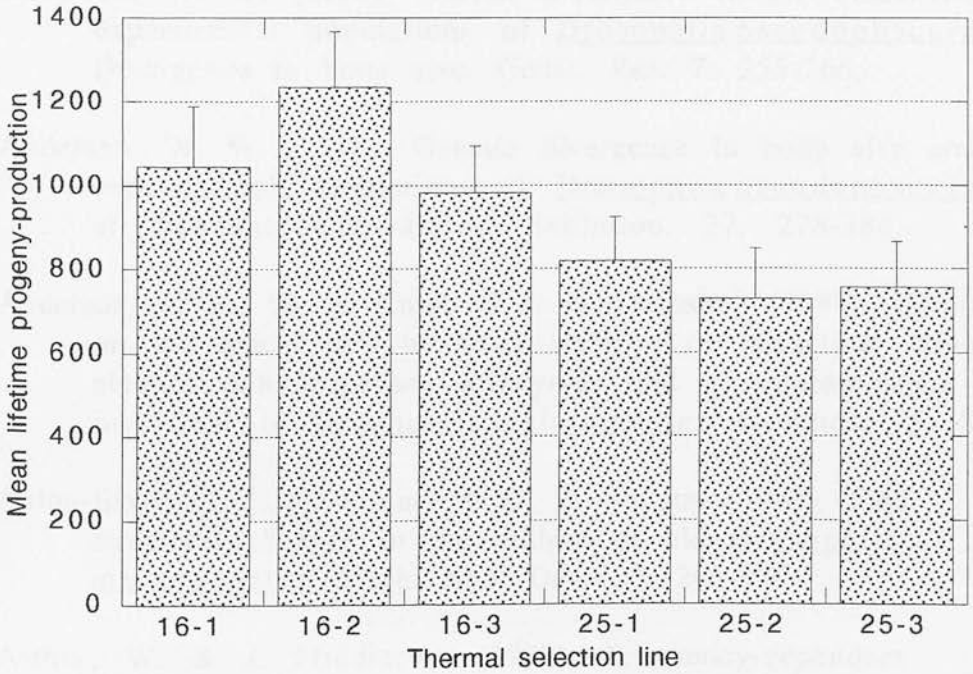


Figure 7.12 Mean lifetime progeny-production for females at 16.5°C. (+/- 95% confidence limits)



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APPENDIX A

APPENDIX TABLE A1 MEAN PUPARIATION TIMES in hours. (+/- 95% Confidence Intervals)

selection temperature	cage line	growth temperature	
		16.5°C	25°C
	1	282.54 (1.18)	121.94 (1.44)
16.5°C	2	276.71 (1.78)	121.17 (1.14)
	3	277.14 (1.87)	122.56 (1.26)
	1	286.84 (1.13)	123.84 (0.77)
25°C	2	284.14 (1.82)	123.69 (0.61)
	3	286.40 (1.74)	123.86 (0.88)

APPENDIX TABLE A2 MEAN ECLOSION TIMES-MALES in hours. (+/- 95% Confidence Intervals)

selection temperature	cage line	growth temperature	
		16.5°C	25°C
	1	514.65 (1.87)	228.17 (1.77)
16.5°C	2	508.11 (2.04)	223.86 (1.16)
	3	508.91 (2.08)	226.46 (0.71)
	1	518.04 (2.39)	223.68 (1.19)
25°C	2	514.20 (2.36)	220.57 (1.18)
	3	520.65 (1.55)	220.62 (1.14)

APPENDIX TABLE A3 MEAN ECLOSION TIMES-FEMALES in hours. (+/- 95% Confidence Intervals)

selection temperature	cage line	growth temperature	
		16.5°C	25°C
	1	504.63 (1.84)	218.22 (1.58)
16.5°C	2	493.63 (2.95)	216.44 (1.59)
	3	493.89 (3.17)	216.26 (1.46)
	1	509.99 (2.13)	216.31 (1.11)
25°C	2	504.96 (2.10)	215.45 (1.34)
	3	511.99 (1.18)	213.25 (0.74)

APPENDIX TABLE A4 MEAN PUPAL PERIOD-MALES in hours.
(+/- 95% Confidence Intervals)

selection temperature	cage line	growth temperature	
		16.5°C	25°C
	1	232.11 (1.25)	106.23 (1.83)
16.5°C	2	231.39 (1.18)	102.69 (1.41)
	3	231.76 (0.97)	105.84 (2.47)
	1	231.22 (2.33)	99.84 (1.25)
25°C	2	230.05 (2.17)	96.55 (1.15)
	3	233.79 (2.78)	96.76 (1.20)

APPENDIX TABLE A5 MEAN PUPAL PERIOD-FEMALES in hours.
(+/- 95% Confidence Intervals)

selection temperature	cage line	growth temperature	
		16.5°C	25°C
	1	222.09 (1.31)	96.28 (1.77)
16.5°C	2	216.92 (1.58)	95.26 (1.45)
	3	216.77 (1.67)	98.04 (2.22)
	1	223.15 (2.02)	92.47 (1.21)
25°C	2	220.82 (1.60)	91.77 (1.62)
	3	225.54 (1.65)	89.40 (0.72)

APPENDIX TABLE A6 MEAN ECLOSION TIMES-MALES in hours.
Larval density=100 per vial.
(+/- 95% Confidence Intervals)

selection temperature	cage line	growth temperature	
		16.5°C	25°C
	1	587.73 (6.06)	228.75 (3.84)
16.5°C	2	584.23 (5.86)	231.58 (3.15)
	3	574.37 (5.08)	230.83 (4.25)
	1	596.07 (6.35)	224.37 (2.36)
25°C	2	602.70 (6.20)	224.26 (2.31)
	3	594.60 (5.87)	224.98 (2.89)

APPENDIX TABLE A7

MEAN ECLOSION TIMES-FEMALES in hours.
Larval density=100 per vial.
(+/- 95% Confidence Intervals)

selection temperature	cage line	growth temperature	
		16.5°C	25°C
	1	575.27 (4.77)	225.85 (3.26)
16.5°C	2	569.37 (4.05)	226.27 (3.25)
	3	564.70 (4.21)	225.29 (3.57)
	1	581.03 (5.37)	221.36 (3.12)
25°C	2	582.77 (5.92)	221.21 (2.94)
	3	581.17 (4.82)	220.60 (2.39)

APPENDIX TABLE A8

MEAN ECLOSION TIMES-MALES in hours.
Larval density=600 per vial.
(+/- 95% Confidence Intervals)

selection temperature	cage line	growth temperature	
		16.5°C	25°C
	1	775.34 (7.94)	356.23 (6.83)
16.5°C	2	780.82 (6.36)	361.03 (5.85)
	3	772.67 (6.17)	358.47 (8.47)
	1	782.23 (6.49)	353.43 (6.47)
25°C	2	783.07 (7.03)	354.10 (4.95)
	3	779.61 (4.79)	350.89 (5.09)

APPENDIX TABLE A9

MEAN ECLOSION TIMES-FEMALES in hours.
Larval density=600 per vial.
(+/- 95% Confidence Intervals)

selection temperature	cage line	growth temperature	
		16.5°C	25°C
	1	765.79 (5.89)	352.67 (6.72)
16.5°C	2	771.17 (2.75)	354.37 (8.50)
	3	768.23 (3.87)	349.63 (4.62)
	1	771.21 (4.74)	351.39 (7.05)
25°C	2	772.83 (5.36)	349.57 (5.43)
	3	770.27 (3.14)	351.52 (6.23)

APPENDIX TABLE A10 Mean number of adults produced from vials Larval density=100 per vial. (+/- 95% Confidence Intervals)

selection temperature	cage line	growth temperature	
		16.5°C	25°C
	1	81.67 (10.04)	79.66 (24.88)
16.5°C	2	89.00 (17.89)	80.33 (22.37)
	3	82.66 (16.19)	76.67 (3.80)
	1	77.00 (10.28)	87.67 (26.56)
25°C	2	75.67 (7.58)	86.67 (7.17)
	3	78.33 (16.16)	88.33 (10.34)

APPENDIX TABLE A11 Mean number of adults produced from vials Larval density=600 per vial. (+/- 95% Confidence Intervals)

selection temperature	cage line	growth temperature	
		16.5°C	25°C
	1	414.60 (9.77)	369.20 (191.79)
16.5°C	2	399.00 (52.11)	381.80 (34.55)
	3	378.00 (38.43)	411.60 (115.15)
	1	390.00 (39.44)	407.6 (40.28)
25°C	2	333.00 (161.41)	397.4 (52.71)
	3	394.00 (86.06)	450.4 (29.45)

APPENDIX B

Appendix Table B1 Larval competitive abilities at 16.5°C

(Data are: n° of wild type adults/n° of wild type adults + n° of sparkling poliert adults x 100)

(a) - low density

DAY	16-1	16-2	16-3	25-1	25-2	25-3
1	35.8	38.0	38.2	28.1	20.7	25.0
2	27.3	38.2	39.7	32.3	23.2	29.4
3	43.6	66.6	37.9	30.3	28.3	29.1
4	44.4	45.3	50.0	14.3	28.1	27.3
5	41.7	33.9	40.0	32.2	29.4	27.8
6	48.2	37.6	38.4	28.3	21.5	24.8
7	41.1	36.4	44.3	30.6	18.9	28.8
8	36.0	42.2	39.1	31.6	24.7	21.4
9	50.1	38.2	41.1	24.6	21.8	27.3
10	32.2	49.1	36.3	25.2	19.8	26.5
MEAN	40.04	42.55	40.5	27.75	23.64	26.74

(b) - Medium density

DAY	16-1	16-2	16-3	25-1	25-2	25-3
1	28.7	27.0	31.4	19.3	21.1	18.4
2	26.6	32.8	23.4	23.9	21.0	19.9
3	26.1	32.8	32.5	22.4	27.6	23.8
4	28.9	35.7	28.2	27.0	27.8	18.8
5	25.0	35.9	19.7	22.5	17.4	20.4
6	30.8	32.6	26.2	20.6	21.2	18.4
7	28.4	26.3	23.8	23.2	28.8	24.9
8	32.2	34.6	28.5	22.1	19.8	24.4
9	26.2	29.3	31.8	28.7	24.2	19.6
10	25.2	33.4	27.9	21.3	19.0	26.7
MEAN	27.81	32.04	27.34	23.1	22.79	21.53

(c) - High density

DAY	16-1	16-2	16-3	25-1	25-2	25-3
1	22.5	31.6	20.8	17.5	18.2	20.8
2	19.9	19.3	22.1	18.2	26.5	22.8
3	23.2	23.3	23.4	18.5	19.4	24.1
4	23.6	24.9	24.6	16.3	22.8	24.0
5	22.8	27.5	18.2	15.1	20.6	21.5
6	23.2	24.8	21.2	18.1	19.6	20.8
7	19.8	22.3	22.1	21.1	23.2	22.8
8	20.0	19.7	22.4	18.5	19.8	21.9
9	23.1	22.8	22.5	20.2	18.9	21.5
10	23.5	24.2	23.1	19.6	23.1	20.8
MEAN	22.16	24.04	22.04	18.31	21.21	22.1

Appendix Table B2 Larval competitive abilities at 25°C

(Data are: n° of wild type adults/n° of wild type adults + n° of sparkling poliert adults x 100)

(a) - low density

DAY	16-1	16-2	16-3	25-1	25-2	25-3
1	23.2	26.6	21.6	32.2	29.8	38.2
2	19.8	24.6	24.7	32.1	36.0	33.9
3	23.2	19.8	18.9	36.3	32.8	34.6
4	27.3	24.8	23.2	38.2	39.7	44.4
5	22.8	26.7	23.4	28.3	39.1	35.7
6	22.3	27.9	23.4	36.2	32.6	35.9
7	14.8	24.8	24.8	29.8	40.0	38.4
8	27.8	23.9	19.4	33.8	36.4	34.2
9	27.8	24.2	24.7	36.1	32.4	39.1
10	26.3	22.8	26.9	31.2	29.8	30.0
MEAN	23.53	24.61	23.1	33.42	34.86	36.44

(b) - Medium density

DAY	16-1	16-2	16-3	25-1	25-2	25-3
1	19.6	19.6	23.1	24.8	28.6	32.5
2	23.9	22.2	23.6	28.8	35.2	26.2
3	22.8	23.6	24.0	24.1	28.4	31.6
4	24.8	21.1	22.4	21.6	28.2	25.5
5	20.2	19.1	25.4	26.7	27.4	32.3
6	22.7	19.5	18.7	28.5	24.1	32.7
7	26.1	22.9	23.7	28.3	30.9	26.7
8	19.3	21.0	18.8	25.6	28.6	28.5
9	23.4	22.5	21.9	26.6	25.7	28.6
10	21.3	17.1	23.4	30.0	28.3	27.4
MEAN	22.41	20.86	22.5	26.5	28.54	29.2

(c) - High density

DAY	16-1	16-2	16-3	25-1	25-2	25-3
1	18.6	23.7	22.4	26.6	28.7	24.7
2	19.6	22.0	21.1	23.8	25.4	24.0
3	15.4	20.8	21.6	29.1	22.0	23.9
4	19.5	20.4	22.4	22.9	27.9	25.7
5	18.2	19.8	21.1	23.4	24.8	24.8
6	18.6	22.3	21.2	24.8	26.4	27.9
7	15.9	22.8	21.0	29.2	22.4	27.1
8	19.1	18.8	22.2	23.4	21.9	24.2
9	21.9	15.8	20.6	25.6	22.4	26.2
10	18.9	19.6	17.2	28.4	25.8	32.2
MEAN	18.57	20.6	21.08	25.72	24.77	26.07

APPENDIX C

APPENDIX TABLE C1 MEAN THORAX LENGTHS - MALES
(+/- 95% confidence intervals)

selection temperature	cage line	growth temperature	
		16.5°C	25°C
	1	26.32 (0.19)	23.54 (0.15)
16.5°C	2	26.66 (0.18)	23.52 (0.18)
	3	26.57 (0.53)	23.78 (0.33)
	1	26.09 (0.19)	23.23 (0.20)
25°C	2	26.23 (0.23)	23.11 (0.22)
	3	26.08 (0.38)	23.26 (0.28)

Data are micrometer units; 1mm=25 micrometer units.

APPENDIX TABLE C2 MEAN THORAX LENGTHS - FEMALES
(+/- 95% confidence intervals)

selection temperature	cage line	growth temperature	
		16.5°C	25°C
	1	30.40 (0.37)	26.67 (0.13)
16.5°C	2	30.61 (0.52)	26.79 (0.28)
	3	30.30 (0.11)	26.86 (0.25)
	1	29.91 (0.16)	26.14 (0.26)
25°C	2	29.74 (0.28)	26.52 (0.27)
	3	29.81 (0.19)	26.15 (0.19)

Data are micrometer units; 1mm=25 micrometer units.

APPENDIX TABLE C3 MEAN WING AREAS - MALES
(+/- 95% confidence intervals)

selection temperature	cage line	growth temperature	
		16.5°C	25°C
	1	2.091 (0.067)	1.540 (0.029)
16.5°C	2	2.089 (0.057)	1.531 (0.019)
	3	2.145 (0.059)	1.549 (0.023)
	1	2.016 (0.077)	1.398 (0.019)
25°C	2	2.003 (0.048)	1.389 (0.0167)
	3	2.013 (0.052)	1.434 (0.027)

APPENDIX TABLE C4 MEAN WING AREAS - FEMALES
(+/- 95% confidence intervals)

selection temperature	cage line	growth temperature	
		16.5°C	25°C
	1	2.544 (0.063)	1.910 (0.061)
16.5°C	2	2.462 (0.059)	1.865 (0.054)
	3	2.564 (0.044)	1.833 (0.040)
	1	2.386 (0.050)	1.815 (0.044)
25°C	2	2.376 (0.040)	1.776 (0.050)
	3	2.359 (0.054)	1.806 (0.044)

APPENDIX TABLE C5 MEAN TRICHOME COUNTS - REGION 1 - MALES
(+/- 95% confidence intervals)

selection temperature	cage line	growth temperature	
		16.5°C	25°C
	1	39.500 (1.152)	48.550 (1.567)
16.5°C	2	38.400 (1.078)	47.450 (1.396)
	3	38.600 (1.228)	48.800 (1.380)
	1	44.000 (1.243)	54.800 (1.612)
25°C	2	40.950 (1.079)	54.050 (1.632)
	3	40.700 (1.086)	52.950 (1.507)

APPENDIX TABLE C6 MEAN TRICHOME COUNTS - REGION 1 - FEMALES
(+/- 95% confidence intervals)

selection temperature	cage line	growth temperature	
		16.5°C	25°C
	1	35.950 (1.507)	46.150 (1.666)
16.5°C	2	35.200 (1.321)	44.300 (0.962)
	3	36.550 (1.632)	45.300 (1.263)
	1	41.600 (1.161)	52.600 (2.040)
25°C	2	43.150 (1.394)	50.200 (1.882)
	3	43.650 (1.149)	46.650 (1.734)

APPENDIX TABLE C7 MEAN TRICHOME COUNTS - REGION 2 - MALES
(+/- 95% confidence intervals)

selection temperature	cage line	growth temperature	
		16.5°C	25°C
	1	53.150 (1.318)	61.250 (1.260)
16.5°C	2	53.600 (1.460)	56.650 (1.786)
	3	51.700 (1.385)	60.600 (1.362)
	1	56.600 (1.483)	66.600 (1.476)
25°C	2	55.650 (1.713)	65.750 (1.440)
	3	55.050 (1.484)	65.900 (1.663)

APPENDIX TABLE C8 MEAN TRICHOME COUNTS - REGION 2 - FEMALES
(+/- 95% confidence intervals)

selection temperature	cage line	growth temperature	
		16.5°C	25°C
	1	46.300 (1.622)	59.300 (1.712)
16.5°C	2	45.050 (1.552)	58.450 (1.977)
	3	43.800 (1.182)	59.700 (1.235)
	1	48.200 (1.182)	68.350 (1.911)
25°C	2	49.300 (1.009)	64.950 (1.948)
	3	48.050 (1.141)	66.550 (1.498)

APPENDIX TABLE C9 MEAN TRICHOME COUNTS - REGION 3 - MALES
(+/- 95% confidence intervals)

selection temperature	cage line	growth temperature	
		16.5°C	25°C
	1	45.200 (1.026)	55.700 (1.804)
16.5°C	2	45.200 (1.338)	58.050 (1.421)
	3	42.500 (1.275)	57.100 (1.510)
	1	47.250 (1.135)	62.950 (1.930)
25°C	2	47.300 (1.138)	63.350 (1.659)
	3	46.900 (1.314)	63.300 (1.629)

APPENDIX TABLE C10 MEAN TRICHOME COUNTS - REGION 3 - FEMALES
(+/- 95% confidence intervals)

selection temperature	cage line	growth temperature			
		16.5°C		25°C	
	1	40.600	(0.556)	52.600	(1.301)
16.5°C	2	40.800	(0.999)	52.700	(1.450)
	3	40.200	(1.056)	51.900	(1.224)
	1	42.750	(0.922)	59.550	(1.912)
25°C	2	44.100	(1.729)	57.900	(1.503)
	3	42.350	(1.126)	58.600	(1.947)

APPENDIX TABLE C11 MEAN TOTAL DORSAL CELL NUMBERS
- REGION 1 - MALES
(+/- 95% confidence intervals)

selection temperature	cage line	growth temperature			
		16.5°C		25°C	
	1	8262.25	(378.973)	7468.75	(223.566)
16.5°C	2	8032.00	(370.651)	7259.45	(211.253)
	3	8281.85	(371.700)	7561.55	(272.548)
	1	8394.30	(431.909)	7655.20	(224.646)
25°C	2	8207.70	(336.448)	7504.65	(242.702)
	3	8199.75	(298.652)	7587.55	(238.269)

APPENDIX TABLE C12 MEAN TOTAL DORSAL CELL NUMBERS
- REGION 1 - FEMALES
(+/- 95% confidence intervals)

selection temperature	cage line	growth temperature			
		16.5°C		25°C	
	1	9148.6	(470.167)	8722.65	(447.615)
16.5°C	2	8660.85	(366.482)	8255.25	(372.525)
	3	9360.9	(399.112)	8312.35	(344.403)
	1	10501.95	(407.970)	9542.35	(429.772)
25°C	2	10244.9	(337.302)	8921.5	(437.960)
	3	10292.4	(329.239)	8420.55	(361.687)

APPENDIX TABLE C13 MEAN TOTAL DORSAL CELL NUMBERS
- REGION 2 - MALES
(+/- 95% confidence intervals)

selection temperature	cage line	growth temperature			
		16.5°C		25°C	
	1	11115.45	(478.146)	9427.45	(216.37)
16.5°C	2	11198.8	(437.027)	8675.8	(331.337)
	3	11084.0	(408.6)	9384.75	(257.152)
	1	11417.5	(553.661)	9312.9	(284.79)
25°C	2	11152.4	(468.815)	9125.45	(191.325)
	3	11066.0	(454.543)	9443.6	(273.641)

APPENDIX TABLE C14 MEAN TOTAL DORSAL CELL NUMBERS
- REGION 2 - FEMALES
(+/- 95% confidence intervals)

selection temperature	cage line	growth temperature			
		16.5°C		25°C	
	1	11765.15	(440.639)	11342.3	(566.72)
16.5°C	2	11092.2	(485.095)	10887.0	(418.629)
	3	11232.6	(390.162)	10944.35	(338.673)
	1	11516.75	(331.374)	12400.55	(451.374)
25°C	2	11710.65	(302.886)	11540.85	(502.657)
	3	11327.6	(316.185)	12007.85	(408.346)

APPENDIX TABLE C15 MEAN TOTAL DORSAL CELL NUMBERS
- REGION 3 - MALES
(+/- 95% confidence intervals)

selection temperature	cage line	growth temperature			
		16.5°C		25°C	
	1	9446.85	(355.764)	8572.75	(289.975)
16.5°C	2	9434.3	(329.428)	8885.4	(252.202)
	3	9128.45	(445.872)	8840.3	(254.595)
	1	9530.55	(460.552)	8795.75	(282.739)
25°C	2	9460.1	(319.638)	8796.75	(262.257)
	3	9436.1	(345.517)	9074.95	(297.246)

APPENDIX TABLE C16 MEAN TOTAL DORSAL CELL NUMBERS
- REGION 3 - FEMALES
(+/- 95% confidence intervals)

selection temperature	cage line	growth temperature			
		16.5°C		25°C	
	1	10327.15	(292.825)	10044.65	(404.504)
16.5°C	2	10032.25	(245.201)	9836.15	(434.241)
	3	10304.95	(294.351)	9515.45	(324.771)
	1	10204.5	(353.254)	10801.35	(406.339)
25°C	2	10474.8	(337.532)	10287.6	(419.441)
	3	10540.65	(245.287)	10565.95	(351.21)